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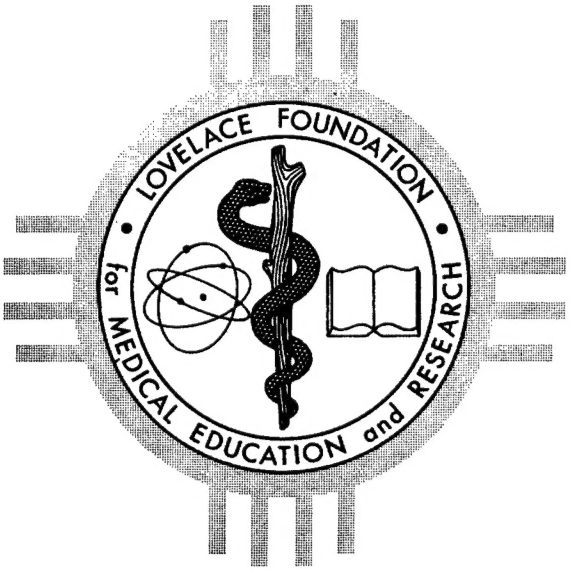

SELECTIVE SUMMARY OF STUDIES ON THE FISSION PRODUCT INHALATION PROGRAM FROM JULY 1964 THROUGH JUNE 1965

Albuquerque, New Mexico

Edited
by
R. G. THOMAS

September 1965

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FISSION PRODUCT INHALATION PROJECT

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SELECTIVE SUMMARY OF STUDIES ON THE FISSION PRODUCT INHALATION PROGRAM FROM JULY 1964 THROUGH JUNE 1965

Edited by
R. G. Thomas

Submitted as a
Technical Progress Report
to
The Division of Biology and Medicine
United States Atomic Energy Commission
on
Contract No. AT(29-2)-1013
September 1965

From the Lovelace Foundation for Medical Education and Research
Albuquerque, New Mexico

UNCLASSIFIED

PREFACE

This report describes work completed or studies in progress on a program aimed at elucidating the biological effects of inhaling fission products. All material contained herein was carried out under Contract AT(29-2)-1013 between the United States Atomic Energy Commission and the Lovelace Foundation for Medical Education and Research, Albuquerque, New Mexico.

Some manuscripts document subtasks which are complete, but are not being considered for publication elsewhere. Others, in the majority, represent work in-progress on continuing projects, some of a long term nature. A complete listing of publications that have appeared as Lovelace Foundation (LF) reports since the inception of this Program is contained in Appendix 1. A similar list containing "open" literature publications is presented in Appendix 2.

ACKNOWLEDGEMENTS

Appreciation is herewith expressed to the individual authors who prepared their respective manuscripts; to Drs. C. S. White, F. G. Hirsch and R. K. Jones who helped editorially, particularly with the more clinically oriented articles; to Mr. Emerson Goff and Mr. Robert Smith for preparation of illustrative material; and particularly to Mrs. Jane Goodrich who, aided by Mmes. Elsie Brehm, Laura Carrasco, Barbara Hahn, Ruth Lloyd, Rita Morris and Nancy Nichols, carried the bulk of the work involving retyping and justifying of edited manuscripts.

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A SIMULATED ANNULUS DETECTOR FOR LARGE VOLUME SAMPLES

by

B. B. Boecker and R. M. Goodrich

ABSTRACT

A gamma ray detection system for biological samples that will yield good spectral data and be insensitive to changes in sample size is being investigated. In this system, the samples are rotated around an axis parallel to the axis of a long, cylindrical NaI detector. Data obtained to date on its response to 1 ml and 1 quart samples demonstrate that this system can be made insensitive to geometry changes of this magnitude while maintaining a usable counting efficiency (3% for Cs-137). Construction details are given. Work on the spectral response is still in progress.

INTRODUCTION

Spectral analysis of biological samples containing mixtures of gamma-emitting isotopes requires a detection system with good resolution and an insensitivity to changes in sample size. A large sodium iodide, NaI, well detector would be satisfactory but the cost is prohibitive. A single crystal system in which the samples are rotated around an axis parallel to the axis of a long, cylindrical NaI crystal was considered a satisfactory and less expensive alternate. In terms of insensitivity to geometry, the end result should be similar to having a stationary sample surrounded by an annulus of NaI. Rotation of the sample should compensate for non-homogeneity and thickness and the use of a tall crystal should minimize the effects of sample height. In addition to its promise as a geometry-insensitive system, a single crystal detector such as this was also favored for spectroscopy work since it would eliminate the need for aligning multiple

detectors.

METHODS

A 3-inch diameter by 8-inch long NaI crystal attached to a premium grade photomultiplier tube (Harshaw Chemical Co., Cleveland, Ohio) was positioned parallel to the axis of rotation of a 5 - inch diameter turntable (18 rpm) as shown in Figure 1. Provision was made for varying the separation, a, between the edge of the turntable and the edge of the crystal and also the distance from the bottom of the crystal to the bottom of the sample, b. A 5-inch diameter lucite cylinder with 1/4-inch walls and 1/2-inch thick base was mounted on the turntable to hold the sample containers. When it was desired to raise the sample in the lucite cylinder, 1 - inch thick pads of rubberized hair packing material were placed between the bottom of the sample and the base of the cylinder until the desired height was obtained.

Both Cs-137 and I-131 were used in these studies. Nine samples of each isotope were prepared in 1 ml vials and counted individually in a Packard well detector (Packard Instrument Co., Downers Grove, Illinois) to insure that equal amounts of activity were in each vial. Eight vials of each isotope were poured into individual quart containers and diluted with distilled water until samples containing the same activity level in volumes of 100, 200, 300, 400, 500, 600, 700 and 800 ml respectively were obtained. These 16 "volume" samples plus the 2 - 1 ml samples were used to determine the characteristics of the system as described below.

RESULTS AND DISCUSSION

A. Large Samples

The quart containers were used to determine the relative effects of a and b on the system's response to changes in sample volume. Values of a = 1.0, 1.5, 2.0 and 2.5 inches were examined with both the Cs-137 and I-131 samples for values of b between 0.5 and 4.75

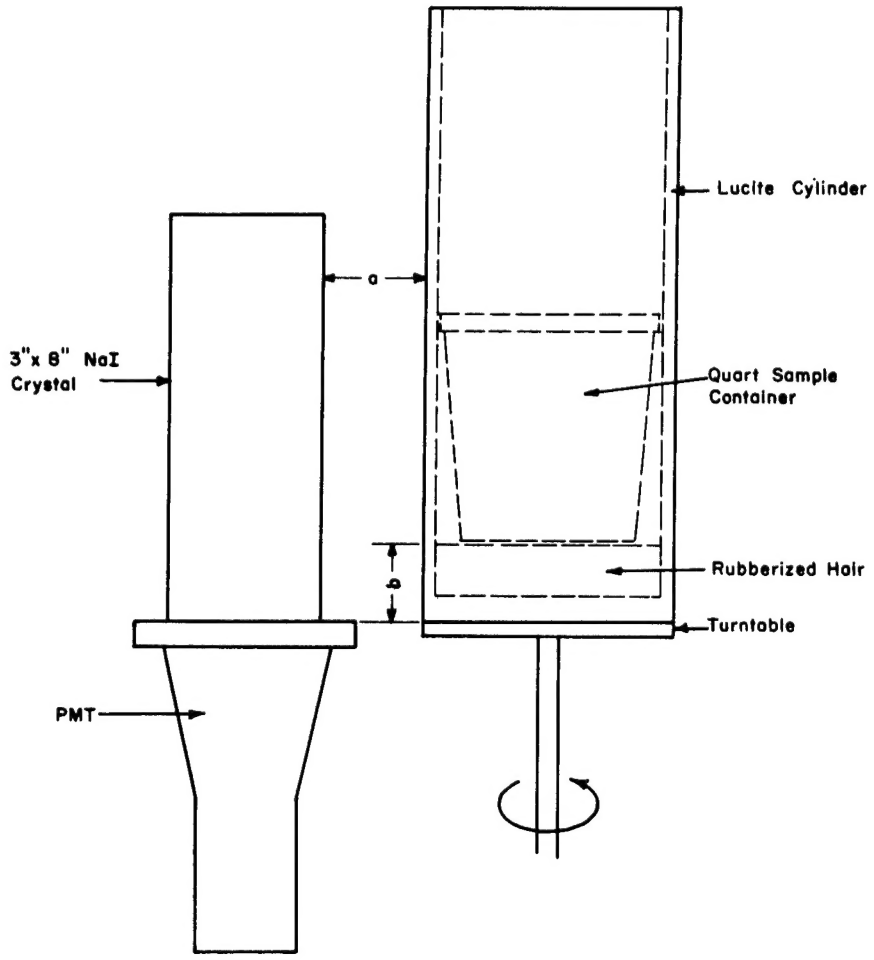


Figure 1: Schematic diagram of the relationship between the 3" x 8" NaI crystal and the turntable.

inches. Typical results with a = 2.0 inches are shown in Figure 2 for Cs-137 and Figure 3 for I-131. A larger change in counting efficiency with sample size was observed for the I-131 samples but the data in both figures are qualitatively similar. When b was small, much of the source activity was concentrated near one end of the crystal. As the source volume was increased, more of the activity was moved closer to the center of the crystal and the observed activity increased. For intermediate values of b, more of the source activity was concentrated near the center of the crystal at all source volumes and changes with increased source volume were minimal. Further increase in b raised the samples to such a position that the larger volume samples approached the other end of the crystal and another decrease in observed activity was obtained.

All of the data of this type are summarized in Table 1 where values for the mean efficiency and the range of values expressed as a percentage of the mean are listed for each combination of a and b used for the Cs - 137 and I - 131 samples. Prior to the start of this work, it was decided that a counting efficiency of about 3 per cent for Cs-137 would be sufficient and, as can be seen in Table 1 this requirement was met for a $\leq$ 2.0 inches. Since some increases in the ranges were observed for values of a less than 2 inches, a value of a = 2.0 inches was considered a practical compromise between efficiency and volume insensitivity.

The selection of a proper value of b depends on the container used since best results are obtained when all samples are located near the center of the crystal. For the quart containers and sample volumes used here, a value of b = 3.0 inches seems to be the best choice.

B. Small Samples

Information on the effect of positioning of small samples was obtained with the 1 ml vials. Each vial was counted at the center of the turntable and also while being rotated in circles at 1 and 2 inch radii. The procedure was repeated at approximately 1-inch intervals along

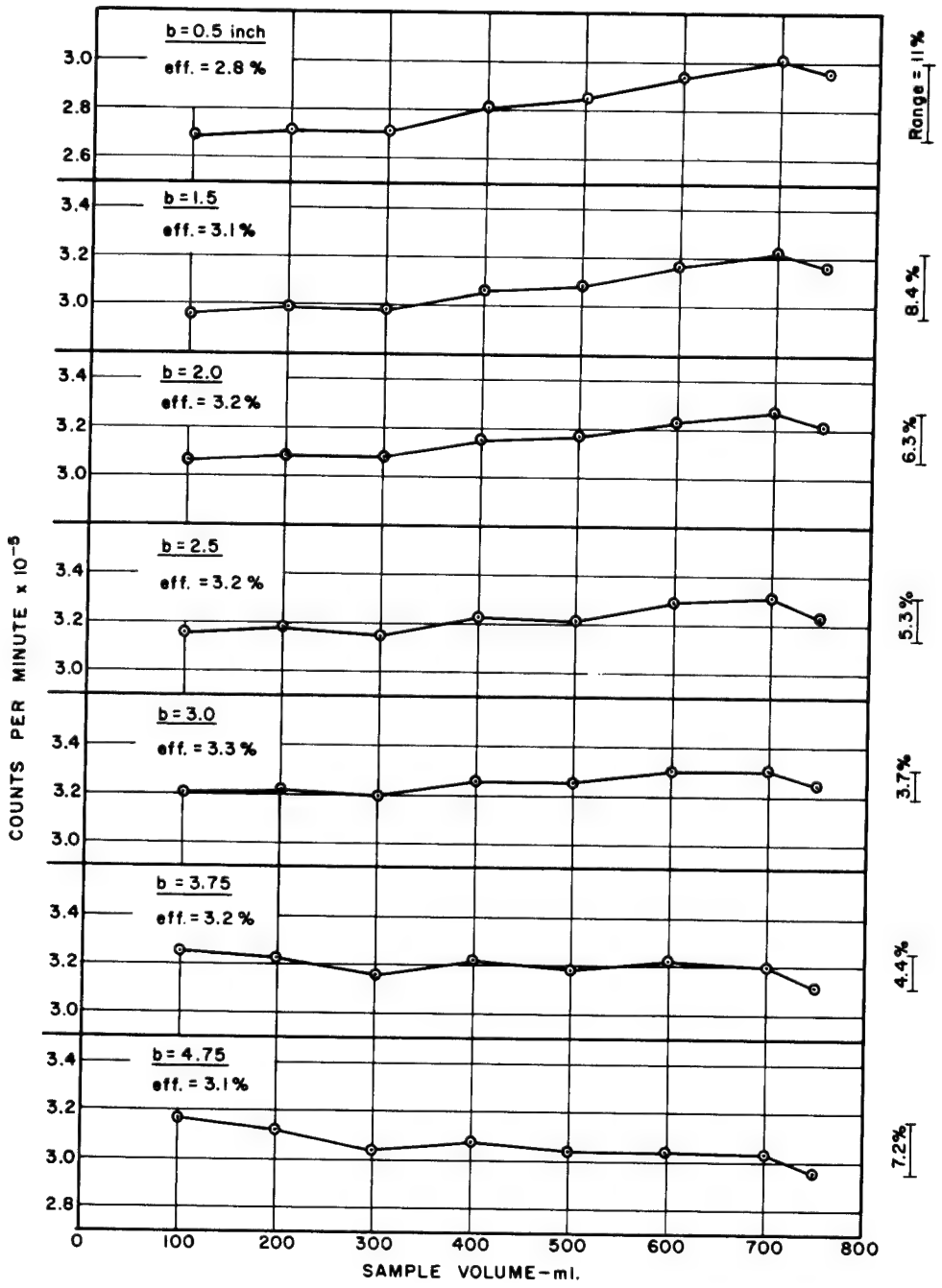


Figure 2: The effect of b on the response of the system to changes in sample volume within a quart container. Results for Cs-137 with a = 2.0 inches.

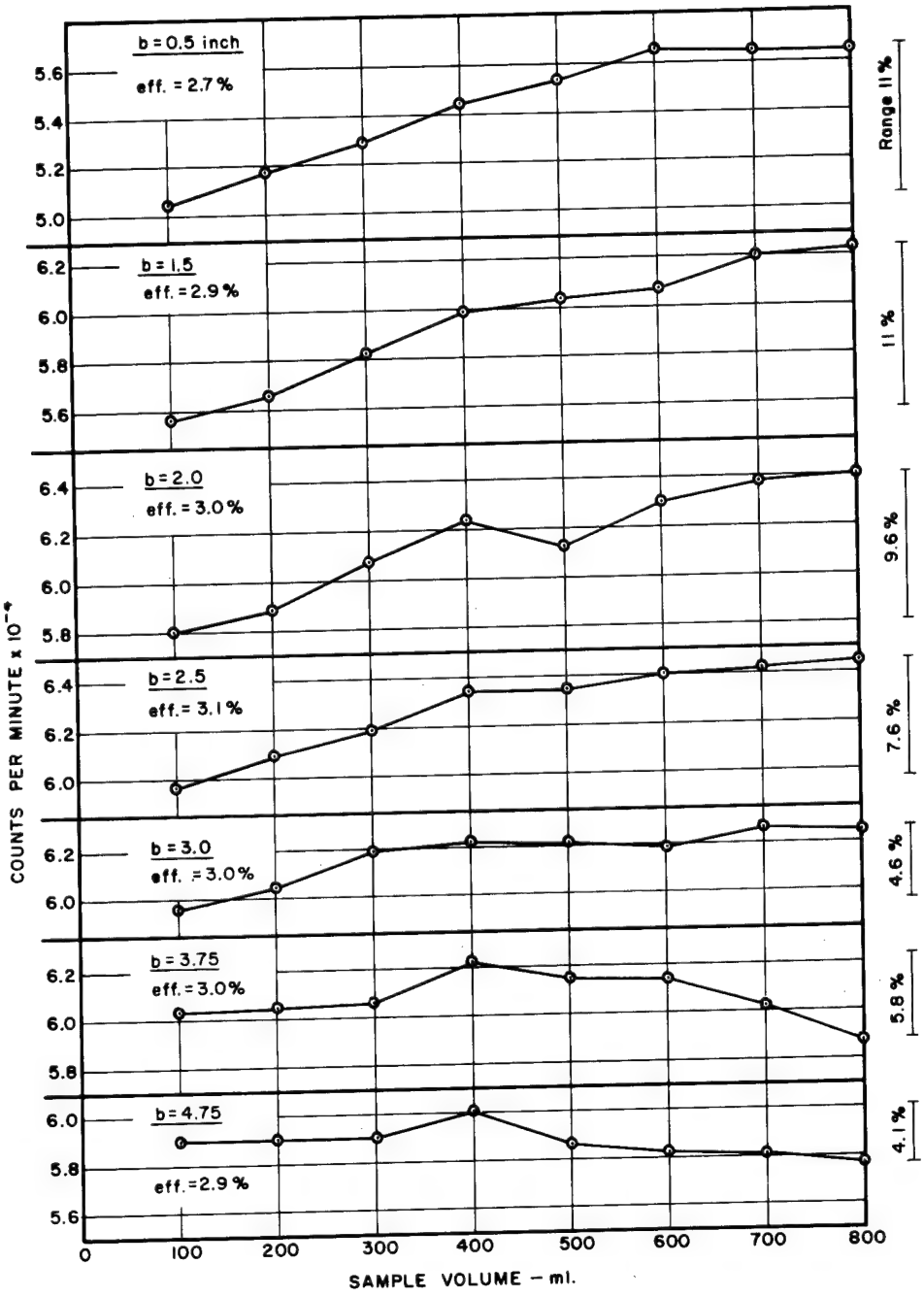


Figure 3: The effect of b on the response of the system to changes in sample volume within a quart container. Results for I-131 with a = 2.0 inches.

Table 1 The Effects of a and b on Range and Efficiency

Cs^{137}

A. Range - %

b in inches	a in inches 1.0	1.5	2.0	2.5
0.5	15.0	12.0	11.0	10.0
1.5	9.7	8.6	8.4	7.3
2.0	7.3	6.9	6.3	6.5
2.5	5.1	5.2	5.3	5.0
3.0	4.0	3.5	3.7	3.9
3.75	5.5	5.2	4.4	4.3
4.75	8.7	8.7	7.2	6.5

B. Efficiency - %

b in inches	a in inches 1.0	1.5	2.0	2.5
0.5	3.7	3.2	2.8	2.5
1.5	4.1	3.5	3.1	2.7
2.0	4.2	3.7	3.2	2.8
2.5	4.3	3.9	3.2	2.8
3.0	4.0	3.7	3.3	2.7
3.75	4.2	3.7	3.2	2.8
4.75	4.0	3.5	3.1	2.7

I^{131}

A. Range - %

b in inches	a in inches 1.5	2.0	2.5
0.5	15.0	11.0	14.0
1.5	12.0	11.0	12.0
2.0	10.0	9.6	9.5
2.5	7.1	7.6	8.6
3.0	6.2	4.6	6.5
3.75	3.5	5.8	2.6
4.75	6.3	4.1	4.5

B. Efficiency - %

b in inches	a in inches 1.5	2.0	2.5
0.5	2.9	2.7	2.3
1.5	3.2	2.9	2.5
2.0	3.4	3.0	2.6
2.5	3.4	3.1	2.6
3.0	3.4	3.0	2.6
3.75	3.4	3.0	2.6
4.75	3.2	2.9	2.5

the height of the crystal by adding layers of rubberized hair between the turntable and the plane of rotation of the source. The results obtained for vials containing Cs-137 and I-131 with $\underline{a}$ = 2.0 inches are shown in Figure 4. In agreement with the data from the quart samples, it can be seen that the observed activity reached a maximum when the source was positioned near the center of the crystal. When either end of the crystal was approached, a drop - off of 20 - 25 per cent in the observed activity was encountered. The middle 3 inches of the crystal appear to respond uniformly and care should be taken to position all sources within this region if possible.

Even though the 1 ml vials used in this study contained the same amounts of activity as the quart containers, the observed activity from them was always less except when they were placed near the center of the crystal and rotated on a circle with a 2-inch radius. When samples in quart containers are being counted, the half of the sample nearest the crystal apparently contributes proportionately more to the observed activity than the other half. Because of this, the same amount of activity in a quart container produces a larger observed activity than the same amount of activity at the center of the turntable. Thus, in order to made a small source count with an efficiency equal to that of a sample in a quart container, it is necessary to rotate it off - center during counting to imitate a distributed source. The exact radius of rotation will have to be determined for each container configuration in order to have all samples counted with the same efficiency.

C. Non-Homogeneous Samples

All of the previous data with quart samples were obtained with homogeneous liquid samples. A homogeneous sample of this type represents a somewhat idealized situation when one considers using this detection system for carcass samples which may contain a mixture of several tissues which contain different concentrations of radioactive material. The localization of all the activity at one point within a quart container represents the worst and also the most unlikely

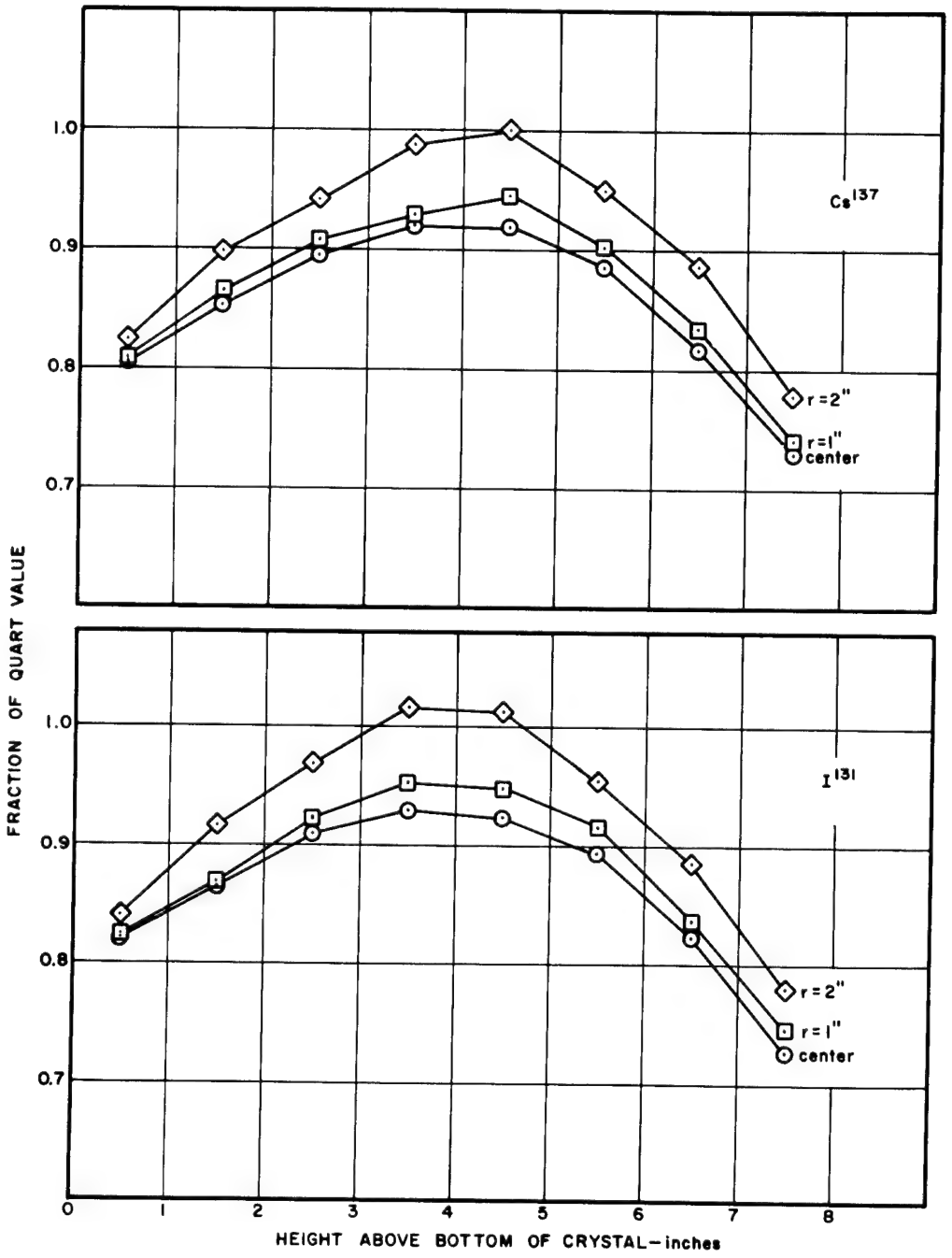


Figure 4: The effect of b and the radius of rotation on the observed activity from a 1 ml vial. All observed activity values are normalized to the activity observed from the same amount of isotope distributed within a quart container.

deviation from the homogeneous condition. If this were to happen, it can be seen from Figure 4 that, at most, this would represent a loss of about 10 per cent in the observed activity if the sample were positioned in approximately the middle 3 inches of the crystal. Since the sample height of a full quart sample is about 3.5 inches, this positioning requirement seems quite feasible.

D. Final Design

From these results, it appeared that this system would fulfill the requirements listed above. Consequently, a shield and cover were designed to facilitate the use and improve the appearance of the system. The detector and turntable were mounted with a separation of $\underline{a}$ = 2.0 inches within a 4-inch thick shield constructed of milled lead bricks and assembled on a dolly for ease of movement (Figure 5). A door mounted on wheels riding on an angle-iron track permits easy access to the sample compartment. When the door is in the full-open position, a microswitch is activated which stops the turntable for sample changing. When the door is in any position other than full-open, the turntable continues to rotate. Spacers that can be placed within the sample chamber to provide the proper radius of rotation and value of $\underline{b}$ will be built for each container configuration.

High voltage for the photomultiplier tube is provided by a 1050-volt battery pack mounted on the underside of the dolly. The 20 rpm motor for the turntable is also mounted below the dolly to keep its associated heat external to the shield. Mounted in this manner, both the battery pack and motor are readily accessible for servicing.

E. Spectral Analysis

All of the data presented to this point have dealt with gross or integral counting. Since this aspect of the system proved to be satisfactory, work is now in progress on the spectral response of the system. With a point source, the resolution of the crystal is about 11 per cent. However, larger samples will produce more scattering and degradation of the gamma rays. Therefore, a considerable amount

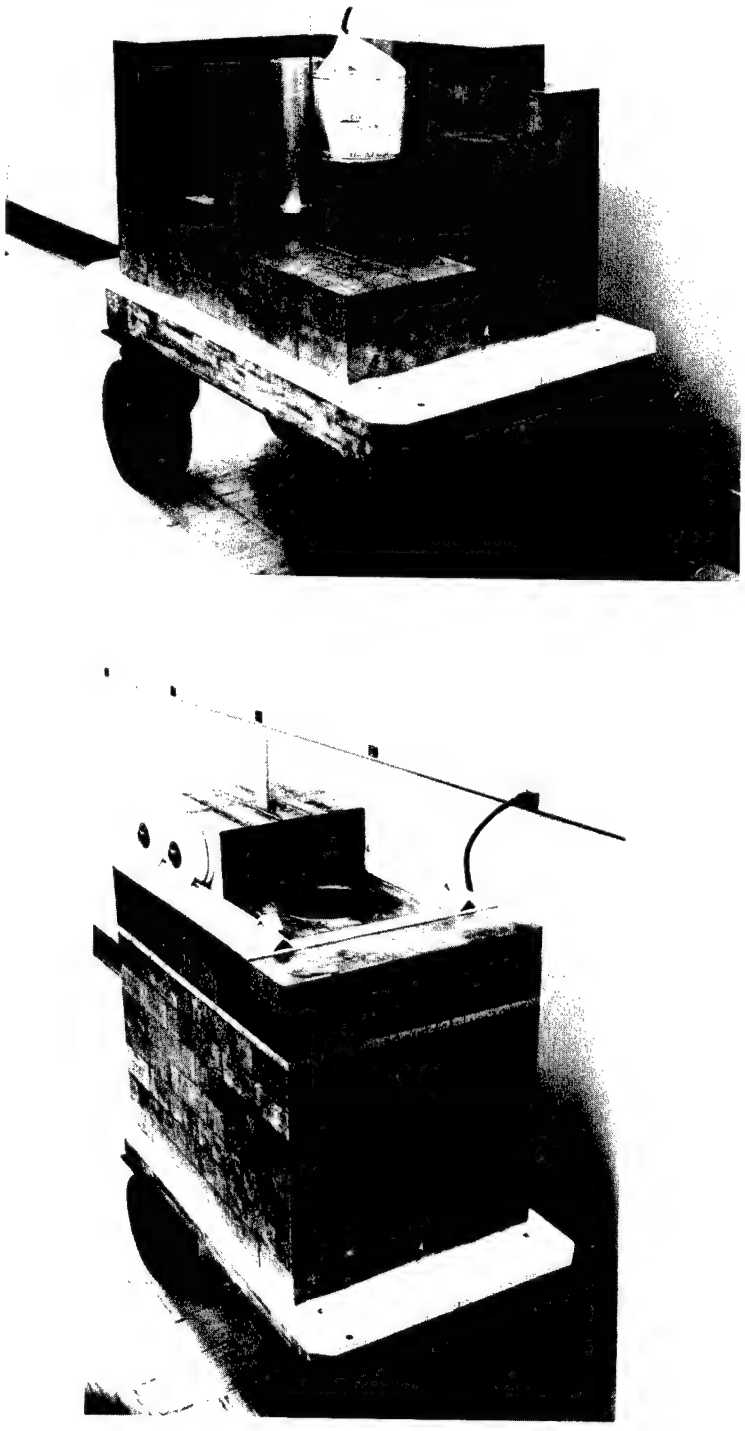


Figure 5: Two views of detector.
(a) Shield partially dismantled with large volume in position for counting.
(b) Shield assembled with cover in place.

of work will be required to define the spectral response versus sample size before this system can be used for routine analysis of isotope mixtures.

A METHOD FOR ASSAY OF HIGH LEVEL SR-90 BY THE BREMSSTRALUNG SPECTRUM

by

G. Newton

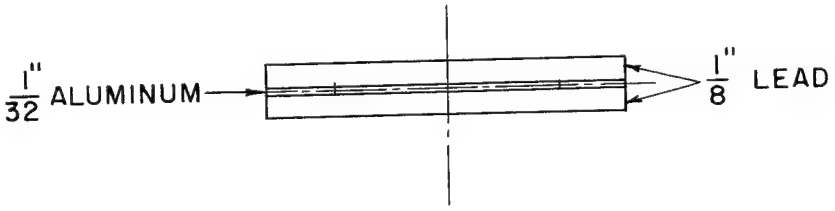
The purpose of this report is to establish a procedure for assay of high level samples of Sr-90.

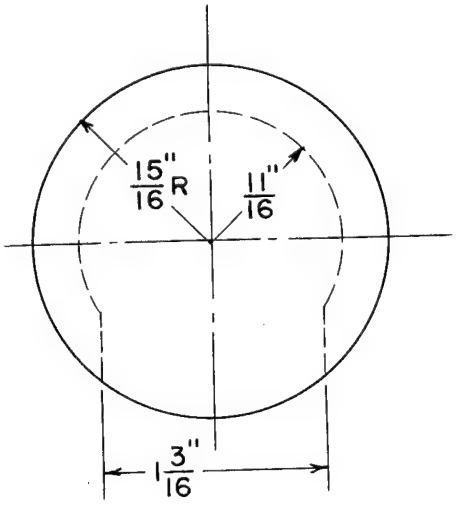
It is possible to assay Sr - 90 by diluting the activity to levels low enough for beta counting, but this method requires that the source be in liquid form. In this Program a method of assay for Sr-90 collected on filters from the animal exposure apparatus was desired. Also, it was anticipated that mixtures of Sr-90 and other radionuclides would be used in the inhalation experiments. Due to the large number of filters used, a rapid practical method of quantitative assay for Sr-90 was necessary. The activity ranges are expected to vary from several millicuries to low microcurie levels.

It was decided that an assay of the bremsstralung spectrum was the most practical solution. Several calibrated sources ranging from 1.0 microcuries to 2.0 millicuries were made. These sources consist of approximately 2 to 10 microliters of solution mounted an 25mm diameter A. A. W. P. Millipore Filters and placed between two pieces of 2.5 mil paper-backed "scotch tape". These sources were then inserted in a 1/8" lead "sandwich" for maximum bremsstralung yield (see Figure 1).

A low level primary standard was made on "thin film" and the activity determined on a 4π beta particle counter. The sample was then placed on a Millipore Filter and taped similarly to the check sources. The primary standard was then beta counted on a CsI counting system and compared to the beta counts obtained from one of the lower level (6.0 microcuries) check sources. These beta counts were made after equilibrium was established between Sr-90 and Y-90.

Figure 1





LEAD SOURCE HOLDER

The source consisting of approximately 6.0 microcuries Sr-90 - Y-90 was selected as the primary bremsstralung standard. Determination of activity on the check sources was done by inserting them into the lead "sandwich" and comparing the bremsstralung counts with the bremsstralung standard. The bremsstralung counts were made on a 3" x 3" NaI crystal connected to a multi-channel pulse height analyzer, a liquid scintillation well-type detector scaler, and on the opposed crystal small animal whole body counter. [1]

On the gamma spectrometer an apparent "peak" in the bremsstralung spectrum was observed at an energy level of approximately 0.27 mev. It was decided to use this "peak" as the reference point for assay. To minimize the effect of gain shift due to differences in count rate, the counts were totaled in several channels above and below the apparent "peak". Due to the activity range of the check sources (10 microcuries to 2.0 millidurie) it was necessary to use several geometries. Source to crystal distances used were: 10, 20, 30, 50 and 100 cm.

Cross checks of the calibration of the reference sources made in the liquid scintillation well-type detector and with the small animal whole body counter, consisting of two opposed 5" x 3" NaI crystals, agreed within ± 5%.

The procedure for assay of high level Sr-90 sources follows:

1. Place a sample of the solution on a filter and tape it so that the source configuration of the calibrated standards is duplicated.
2. Place the mount of the unknown activity in a lead "sandwich" and count with a gamma spectrometer.
3. Select a calibrated source of approximately the same activity, place in a lead "sandwich" and count in the gamma spectrometer, using the same geometry and gain settings.
4. Compute the activity by comparing the total counts found in the significant channels of both the unknown source and the calibrated source.

An alternate method would be to compare total counts as determined with any suitable scintillation counter. If other radioisotopes are present, the preferred method would be by a gamma spectrometer and least squares analysis. This method utilizes principles as described in Applied Gamma-Ray Spectrometry by C. E. Crouthamel, Ed., Pergamon Press, New York, 1960.

REFERENCES

1. Goodrich, R. M., R. G. Thomas and S. R. Wright, "A Gamma Ray Detector of Variable Geometry for Whole Body Counting of Small Animals," Lovelace Foundation Report, LF-14, 1964.

CROSS CALIBRATION OF CsI CRYSTAL COUNTERS

by

S. Posner

INTRODUCTION

Cross calibration to evaluate efficiencies and "dead-time" factors for three counting systems in use by the Aerosol Physics Department was necessary since the data from these systems are used interchangeably.

The three counting systems are:

1. A 4π counter utilizing methane gas and Radiation Instrument Development Laboratory (RIDL) components.
2. A 2π Nuclear Measurements Corporation (MNC) No. 3 PC P-10 gas counter.
3. A 1 inch x 5 millimeter CsI crystal used with RIDL counting components.

PROCEDURES

Several 4π films of each nuclide of interest were prepared. Those nuclides which required time to reach equilibrium after preparation were stored in a vacuum bell jar for a proper time interval before counting. After 4π counting, half of the films were transferred to filter backings and half to 22 mm. glass cover slips. This was necessary to simulate actual age when samples from inhalation exposure units are collected on glass cover slips.

Both "cover slip" samples and MPAA samples were counted on the 2π counter and the CsI counter and then recounted with the faces taped with paperbacked tape. This was done to simulate actual use when the exposure chamber filters and cascade impactor cover slips are "sandwich"

taped. These studies were attempted to evaluate the efficiency loss due to the "taped sandwich".

EXPERIMENTAL WORK

The actual physical experiment was quite elementary and was performed as follows:

1. 4π counts were performed on the sample;
2. Sample films were transferred to either a filter or a cover slip;
3. Samples were counted "open face" taped onto the CsI crystal;
4. Samples were counted "open face" taped into the 2π counter;
5. Efficiency was computed for 4π versus CsI counters and 4π versus 2π counters;
6. Dead time factors for the 4π and 2π counters was computed and the proper correction was plotted.

RESULTS AND CONCLUSIONS

Based upon the data in Tables 1 and 2 the following conclusions were made.

1. Paperbacked tape covering samples may effect 2π efficiencies by 10 to 45 per cent.
2. The effect upon efficiency of filter backing versus glass backing is significant for certain nuclides of interest to this Program, particularly Cs-144, Sr-90 and I-131.
3. Effect of "tape sandwich" on counting directly with CsI crystals is not significant.
4. Dead time factors on both 2π and 4π counters are quire important.

Table 1

PER CENT EFFICIENCY*

Isotope	Open Face		Tape Sandwich	
	CsI	2π	CsI	2π
Y-91	2.7	65.0	2.7	51.6
Ru-106	2.3	56.7	2.4	42.9
Sr-90	2.4	63.5	2.5	49.3
I-131	1.7	66.3	1.55	30.9
Co-60	-	58.4	-	14.7
Ce-144	-	60.1	-	39.8
Ce-144	-	52.1	-	31.8
Cs-132	1.8	62.8	1.6	33.1

*Average of four films, both glass and filter backing.

Table 2

PER CENT EFFICIENCY

	Glass Backing				Filter Backing			
	Open Face		Tape Sandwich		Open Face		Tape Sandwich	
Nuclide	CsI	2π	CsI	2π	CsI	2π	CsI	2π
Y-91	2.8	65.2	2.8	51.3	2.55	64.6	2.65	51.8
Ru-106	2.35	55.0	2.4	42.5	2.25	57.2	2.3	43.2
Sr-90	2.55	65.0	2.67	49.5	2.25	60.0	2.25	48.0
I-131	1.8	66.0	1.6	31.8	1.6	66.0	1.5	30.0
Co-60	-	60.1	-	16.0	-	57.0	-	13.5
Cs-137	1.9	64.0	1.6	34.0	1.7	63.0	1.6	32.0
Ce-144	-	67.0	-	40.0	-	54.0	-	39.8

A COMPUTER PROGRAM FOR ANALYZING ANIMAL HISTORIES

by

R. W. Albright, L. H. Mallory and W. R. Kerzee

A. Objectives

This computer program is designed:

1. To be a long term depository for all medical treatment and observational data accumulated on individual animals.

2. To help with the routine functions of animal colony management by:

   a. scheduling routine work;
   b. controlling work in progress;
   c. reporting facility activities.

3. To retrieve and analyze data of interest to the Research and Veterinary Staff in estimating population parameters for normal and experimental animals.

4. To retrieve appropriately selected sections, or complete individual animal histories, for the Department of Veterinary Medicine, or for inclusion in interdepartmental reports.

B. Accomplishments to Date

Data acquisition has been organized to completely parallel the normal flow and content of information required by the Department of Veterinary Medicine. Work orders initiated as instructions to caretaker and technical personnel are typed in stylized format on a Friden Flexowriter. Results from work initiated by work orders, and reports of other work accomplished, are similarly prepared. A stylized format for data preparation has been designed to require a minimum of coding at the

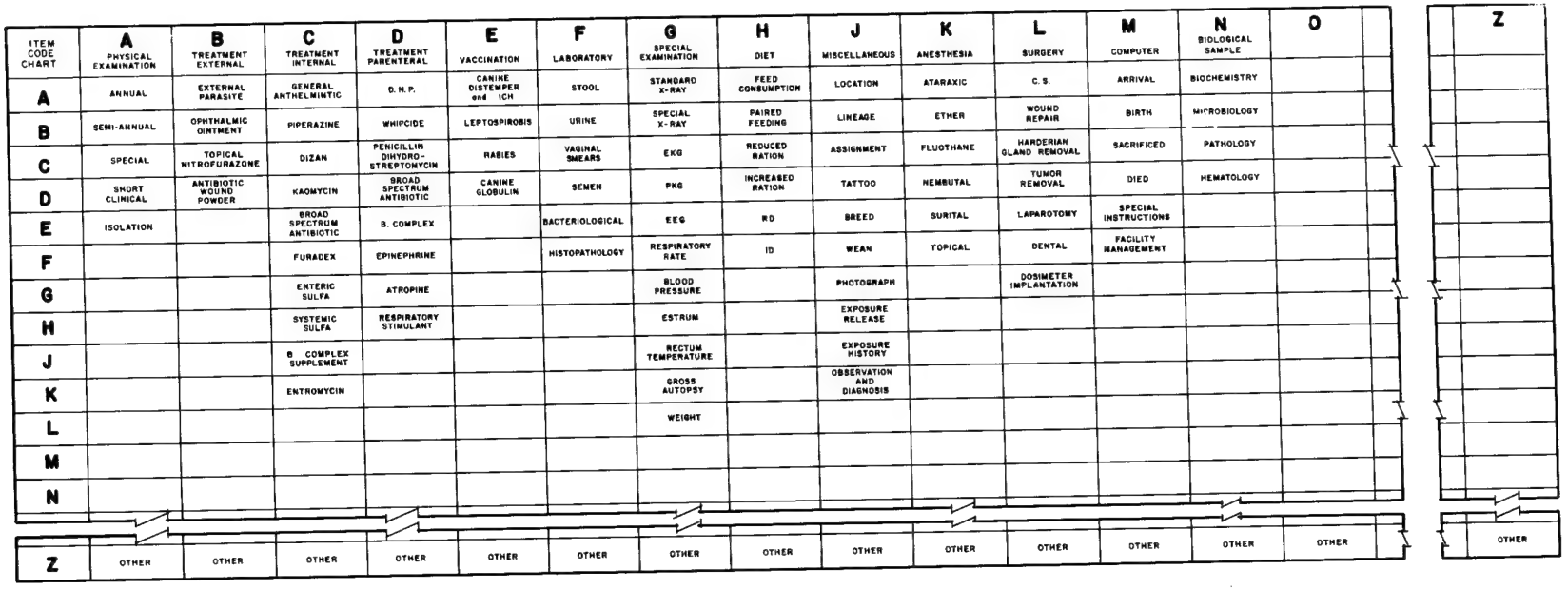
origin and yet to incorporate all the features required for automated storage and retrieval. (See Item Code Chart, Table 1.)

Acquisition of the work order information for computer processing is accomplished by daily input of punched paper tape from the Flexowriter. Medical records pertaining to the canine colony are extracted, edited, processed and added to the current and permanent history files, which have been generated by the computer facility. By updating the medical history files daily, for subsequent sorting and merging with previously accumulated medical histories, the Department of Veterinary Medicine is able to realize a short "turnaround time," thus improving the efficiency of operations.

Simultaneously with the updating of animal histories, information from the history file is collected for issuing work orders, for routine or periodic examinations or observations due for scheduling, and for reporting work orders till incomplete and overdue.

C. Present Status

Implementation of the initial phase of this system, on objectives 1 and 2, is complete. The remainder of the system, objectives 3 and 4, will be implemented in FY-1966. Much of this is still in the planning stage and will require more definition in order to design the automated system.



ITEM CODE CHART	A PHYSICAL EXAMINATION	B TREATMENT EXTERNAL	C TREATMENT INTERNAL	D TREATMENT PARENTERAL	E VACCINATION	F LABORATORY	G SPECIAL EXAMINATION	H DIET	J MISCELLANEOUS	K ANESTHESIA	L SURGERY	M COMPUTER	N BIOLOGICAL SAMPLE	O	Z
A	ANNUAL	EXTERNAL PARASITE	GENERAL ANTHELMINTIC	D. N. P.	CANINE DISTEMPER and ICH	STOOL	STANDARD X-RAY	FEED CONSUMPTION	LOCATION	ATARAXIC	C. S.	ARRIVAL	BIOCHEMISTRY		
B	SEMI-ANNUAL	OPHTHALMIC OINTMENT	PIPERAZINE	WHIPCIDE	LEPTOSPIROSIS	URINE	SPECIAL X-RAY	PAIRED FEEDING	LINEAGE	ETHER	WOUND REPAIR	BIRTH	MICROBIOLOGY		
C	SPECIAL	TOPICAL NITROFURAZONE	DIZAN	PENICILLIN DIHYDRO-STREPTOMYCIN	RABIES	VAGINAL SMEARS	EKG	REDUCED RATION	ASSIGNMENT	FLUOTHANE	HARDERIAN GLAND REMOVAL	SACRIFICED	PATHOLOGY		
D	SHORT CLINICAL	ANTIBIOTIC WOUND POWDER	KAOMYCIN	BROAD SPECTRUM ANTIBIOTIC	CANINE GLOBULIN	SEMEN	PKG	INCREASED RATION	TATTOO	NEMBUTAL	TUMOR REMOVAL	DIED	HEMATOLOGY		
E	ISOLATION		BROAD SPECTRUM ANTIBIOTIC	B. COMPLEX		BACTERIOLOGICAL	EEG	RD	BREED	SURITAL	LAPAROTOMY	SPECIAL INSTRUCTIONS			
F			FURADEX	EPINEPHRINE		HISTOPATHOLOGY	RESPIRATORY RATE	ID	WEAN	TOPICAL	DENTAL	FACILITY MANAGEMENT			
G			ENTERIC SULFA	ATROPINE			BLOOD PRESSURE		PHOTOGRAPH		DOSIMETER IMPLANTATION				
H			SYSTEMIC SULFA	RESPIRATORY STIMULANT			ESTRUM		EXPOSURE RELEASE						
J			B COMPLEX SUPPLEMENT				RECTUM TEMPERATURE		EXPOSURE HISTORY						
K			ENTROMYCIN				GROSS AUTOPSY		OBSERVATION AND DIAGNOSIS						
L							WEIGHT								
M															
N															
Z	OTHER	OTHER	OTHER	OTHER	OTHER	OTHER	OTHER	OTHER	OTHER	OTHER	OTHER	OTHER	OTHER	OTHER	OTHER

Table 1

Item Code Chart

A COMPUTER PROGRAM FOR ANALYZING DISTRIBUTION, EXCRETION AND RETENTION DATA

by

R. W. Albright

Acquisition of counting data from the automated radioactivity determination system has been a reality for almost two years. Data generation in the first full fiscal year of operation has achieved about one third the rate anticipated at optimal operation. Approximately 200,000 counting records were collected during this interval, about 70 percent being from excreta and tissues obtained with the automated readout system. Although an additional readout system for small animal whole body counting has been acquired, it is not yet operational for routine recording of experimental data.

Punched paper tape from the automated data acquisition system is currently being forwarded to the computer facility at two or three day intervals. A complete listing of these records, as stored in the computer, is returned immediately to the counting laboratory for editing. (This phase of the operation has been found necessary because the reliability of this system has been below desirable levels.) Corrections are listed on data sheets and returned to the computer center for keypunching within a week of the counting. As previously mentioned, these records comprise the entirety of the excreta and tissue counting data from the Fission Product Inhalation Program.

Whole body counting data are accumulated in counting books throughout a week and are sent to the computer facility. The record sequencing conventions for both modes of data collection are identical, as is the record format. This not only facilitates data processing but achieves a uniformity of operation in preparation for the forthcoming automation. Keypunching of this information is normally accomplished within two days, roughly coinciding with completion of preparation of corrections to the

paper tape records already entered for the previous week.

Since the whole body counting data requires no pre-editing to eliminate errors similar to those introduced by the automated system, a week's records can be transferred to the counting data file with corrections being simultaneously incorporated; and permanent, sequential identification numbers can be assigned. At this time a listing with the permanent identification numbers is printed. Any further correction of records (for a given counting date) requires reference to these identification numbers; and any correction results in preparation of a new listing which reports only the current records for that date. This counting data file is considered the primary data file, and it is safeguarded from inadvertent destruction.

Continuing with the description of the initial weekly processing of counting data, all experimental records for those dates are extracted from the entire collection (which contains calibration and background counting also); and are completed with the counter constants (background rate and counter efficiency), for the appropriate counting unit as well as the isotope decay rate constant. Also, the counting date, collection date for excreta specimens and sacrifice data for animal tissues are reduced to day post-exposure records for the particular experiment. The required form for corrections for decay is applied at a later date, as well as that for ordering the experimental records. Data checking for a variety of conditions required for valid processing in later reduction is made at this time, and diagnostic messages for those records with detected errors are printed. After sorting into order by experiment number, animal (or cage) number and day post-exposure (or tissue number) a listing for each cage or animal active in this period is also prepared. This provides a quick visual check of information for internal consistency on a day-to-day basis. An additional correction cycle is now performed, identical to the above but instead of reproducing the printed listing for each experimental cage or animal, the information is merged into the records to await completion of the experiment. The records accumulated for a given experimental cage or animal are reduced and analyzed at the request of the

experimenter.

The first six months of FY-65 were mostly concerned with processing the backlog of counting records which had accumulated before the B-5000 computer configuration was completed. This latter has a punched paper tape input with adjusting operating procedures and programs to correct, or circumvent, errors introduced by malfunction of the automated read-out system. Since interpretation of each counting record is dependent upon information within the record itself, file maintenance problems of an unpredictable nature arose which greatly exceeded those which had been anticipated. A minor redesign of some record structures was required to accommodate the expanded data ranges when information from dog exposures was phased into the program. This necessitated the introduction of a pre-editing phase for records from the automated data collection system. The basic processing procedure, as designed remained essentially unaltered. Other record modifications are also needed in order to eliminate restrictions in operating procedures at the counting laboratory. These changes will preclude ambiguity in data identification, and consequently improve data quality. Due to the considerable program maintenance required to effect these alterations, however, they have been deferred until the requirements for computer processing of multiple isotope counting data will have come into being.

During the second half of FY-65 reduction of experimental data was of primary concern. Normally the daily counting records for any animal cage in retention experiments, consists of counts from; 1) an empty, clean cage, 2) a clean cage after animal transfer, 3) urine collected, and 4) feces collected. Provision for combining partial samples of urine or feces, adjusting for several days excreta combined into a single sample and, in the case of dogs, an experiment to correct counter efficiency as a function of sample weight are included in the reduction program. Also, if recleaning of a cage is required because of excessive contamination, only the final empty cage count is accepted. For any experimental record the following steps are required; 1) computation of counts per minute, 2) subtraction background count rate, 3) testing for significance of sample

count rate above background count rate, and 4) conversion of net count rate to disintegrations per minute. For excreta samples, corrections are made for isotope decay from date counted to date collected.

After processing the records for a single day the empty cage radioactivity is subtracted from that of the full cage and the ratio of the two is computed. The printed report also shows these quantities after correction for decay to day of exposure. To obtain the ratio of radioactivity in the excreta to the whole body, excreta rates are divided by the number of days which had elapsed since the last sample was collected. The printed report also gives the comparable values after correction for decay to day of exposure. Also, the urine and feces disintegration rates after correction to day of exposure are accumulated to give the total radioactivity accounted for in excreta for the duration of the experiment.

Tissue records for a given experimental animal are reduced in a similar way and samples of canine tissue again require a counter efficiency correction for sample weight. For each tissue the total radioactivity, radioactivity per gram, percent of initial whole body burden and percent of initial whole body burden per gram are corrected to date of sacrifice, and to date of exposure, for presentation on the printed report. The reduced data, disintegrations per minute corrected to day of experiment for retention and excreta and to day of exposure for tissues, are merged into a file of experimental results which summarizes all information obtained. These are used as input for all subsequent analyses.

The data reduction program described above has had considerable productive usage. Also operational, but not yet finally integrated is the sum of exponential curve fitting for retention data.

A preliminary program for reduction of background and calibration counting data with graphical presentation was also completed in FY-65. The design was unfortunately of a restricted form and lacked the generality and flexibility required for analysis of calibration data, where complete characterizing parameter values were not available in advance of reduction. It appears that reduction may require analysis analogous to retention

experiments for estimating the half-life of an isotope from empirical data. If calibration data are to be useful in computing counter efficiencies, the study of a particular standard must precede reduction of experimental counting records referred to it.

Completion of programs required for all analyses mentioned should be operational before the end of the current calendar year. It appears that the redesign specifications for acquisition and reduction of multiple isotope exposure data will also be concluded. Thus computer program design and implementation can be started early in 1966. Of course, the necessity for maintaining compatibility with data collected prior to conversion to the B-5000 will pose problems not previously encountered; as will the requirement for continuous processing of information throughout the transition period.

Also planned for early 1966 is the automated acquisition of respiratory function data for all small animal inhalation exposures. Data will be placed on magnetic tape which will be transported to a central analog to digital conversion system at the computer center, for subsequent reduction on the digital computer. At the same time simplified data collection forms for other experiments will be implemented.

A PATHOLOGY REQUISITION-REPORT FORM FOR EXPERIMENTAL USE WITH COMPUTER CODING

by

T. L. Chiffelle, R. K. Jones and J. K. Scott

A combined requisition-report form has been devised for general use in laboratories where tissues are submitted to a central pathology laboratory for evaluation. The ultimate purpose of the form was:

1. To furnish all necessary information concerning an animal experiment, including all experiment numbers, animal numbers, type of experiment, pertinent clinical information, and the gross examiner's findings.

2. To serve as a convenient system for cataloging and compiling results within the pathology department.

3. To supply a prompt and readable resume´ of the anatomic findings to the requesting laboratory.

4. To provide a system of "open-ended" coding of anatomic findings suitable for central computer input.

In order to encourage complete submission of important numbers and other experimental information by the examiner, the form was designed to provide for convenience and simplicity in use. A uniform entrance of bits of information is desirable, especially when animal dissections or tissue samplings are performed by many individuals of several laboratory sections.

The use of computer memory storage and retrieval has become a valuable and time saving method of integrating and summarizing results from several laboratories engaged in the same series of experiments. Initially, in our institution, all animals acquired are assigned reference

numbers (by the Department of Veterinary Medicine). These are not duplicated and conveniently serve as a master index number for computer data storage. For internal convenience, however, individual laboratories usually assign their own experiment and accession numbers which have meaningful significance to the series being dealt with. For efficient use of computer storage, however, the latter numbers are related to the original primary reference number assigned to the animal.

Some forms of analytical data, such as biochemical analyses on blood sera are readily reduced to a system of numerical values which can easily be stored on computer tape. The problem of converting pathologic descriptions and anatomic diagnoses to a code suitable for retrieval and categorization is difficult to solve, unless the range of anatomic variations and pathologic diagnoses can be restricted. However, when the limits of possible variations are unpredictable, the task of designing a computer code system of sufficient flexibility or "open-endedness" becomes formidable.

The recently published Systematized Nomenclature of Pathology (SNOP-Code) by the Committee on Nomenclature and Classification of Disease of the College of American Pathologists (First Edition, 1965) seems to have overcome much of the difficulty in coding and classifying pathologic diagnoses. This well organized classification has been developed over a six year period, and has had extensive trial evaluations in many laboratories. Primarily designed to assist in organizing and utilizing medical pathologic material, the coding system has sufficient flexibility to be of significant value in storage and retrieval of data from research laboratories. In effect, diseases are defined into four general areas of information (Topography, Morphology, Etiology, and Function), groupings which reflect certain natural relations. Within any one of these four groups, terms are assigned a four-digit number. The most useful feature, particularly to the experimenter, is the flexibility, or "open-endedness," either between individual items, or between narrow subclassified categories. Thus, the code is capable of being continuously expanded to meet the needs of advancing knowledge.

To increase this flexibility two simple modifications have been locally introduced. The first is to expand the four-digit entries in each group to five digits, thereby increasing the limits of new entries between each basic number by a factor of ten. In addition, we have added a fifth general category with a designation "Phase Modifier" into which another four or five digit numbering code is entered. This latter code number may be further subdivided into twin two-digit, or into two and three-digit systems designed to give free expression to the anatomist in qualifying the magnitude of the pathologic changes, or the distribution of the lesions which he finds. This is particularly important when the interpreter is concerned with quantitating the observed changes. The details of the fifth categorical classification can rather easily and arbitrarily be established by the individual experimenter to suit his particular needs. Depending on whether a four or five-digit number is chosen for this latter group, a choice of 1,000 to 10,000 qualifying entries is possible. This is probably more than sufficient for most needs. An example of a useful phase modification code is presented below.

PHASE MODIFIER-P

5th Category			
0 0		0 0	
00	NOS*	00	NOS
01	Focal	01	Questionable significance
02	Multiple	02	Trace
03	Diffuse	03	Mild
04	Acute	04	Moderate
05	Subacute	05	Severe
06	Chronic	06	Small (< 30μ)
07	Early repair	07	Medium (30μ to 100μ)
08	Late repair	08	Large (>100μ)

*NOS: Not otherwise specified.

The left-hand twin-digit column qualifies the nature and/or distribution of the lesions; the right twin-digit column denotes degree or dimensions of lesions. By extending the classification of each twin column, one can

cross-reference the items listed (by example) in each. Thus, a lesion of multiple distribution in an organ or tissue can be designated as "acute, subacute, or chronic, etc." To avoid confusion, the established arbitrary code should be acceptable and understood by all sections which use it. It should be noted that in some sections of the SNOP-Code, phase modifiers have already been coded and entered into the four-digit system of the 4 categories. This applies particularly to the many types of inflammation. However, there are other types of coded changes which have no such means of expressing degree or magnitude of change. Thus, the fifth and last group provides the examiner with this expression. No attempt will be made to elaborate upon the details of handling the SNOP-Code in the first 4 categories since this has already been well described in the introduction to the published volume. One further advantage of the SNOP-Code is that perhaps for the first time, pathologic diagnoses and descriptions can be given a uniform code number which will be understood by a variety of laboratories.

To facilitate in the dissection and description of the various tissues submitted, the combined "Requisition-Report" form was designed as shown in Figure 1. The final form resulted from many trial variations. Many factors were evaluated to suit the needs of the laboratories concerned. Essentially, it is divided into four major sections. The first, or top four lines, document the identification numbers of the animal, laboratory code, date, species, age, sex, and experiment. The pathology index code number and the accession number are added after the material has been received by the Pathology Department. All other information on the form is supplied by the originating laboratory. The second section (Examiner's Findings and Comments) provides for the gross examiner's positive findings, and any other comments pertinent to the clinical condition of the animal, or to the nature of the experiment which may be necessary for interpretation. At the extreme right side of the form, running vertically, a check-off list is provided to indicate the tissues or samples submitted. Special items not included in the list may be designated at the

PATHOLOGY

Animal / Vet. Med. Number	Experiment Code Number	Path. Series Index Number	Pathology Asc. Number	Species:	
				Age:	Sex:

Date		Originating Laboratory	Isotope	After Days Exposure		Sacrifice Series		Died
Year	Day of Year							
			Dose			Longevity Series		Sacrif.

EXAMINER'S FINDINGS AND COMMENTS:

(USE REVERSE SIDE FOR ADDITIONAL SPACE)

PATHOLOGY DIAGNOSIS:

SNOP CODE

	T	M	E	F	P
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

- Skin
- Breast
- Thymus
- Lymph Nodes
- Spleen
- Blood
- Marrow
- Bone
- Muscle
- Adipose
- Larynx-Trachea
- Bronchus
- Lung
- Heart
- Blood Vessel
- Teeth
- Tongue
- Salivary Gld.
- Tonsils
- Esophagus
- Stomach
- Duodenum
- Ileum
- Colon
- Liver
- Gall Bladder
- Pancreas
- Kidney
- Ureter
- Urinary Blad.
- Urethra
- Prostate
- Seminal Ves.
- Testes
- Vagina
- Uterus
- Fallopian T.
- Ovary
- Placenta
- Fetus
- Pituitary
- Adrenal
- Thyroid
- Parathyroid
- Paraganglia
- Brain
- Nerve
- Eye-Orbit
- Ear-Mastoid
- Sinuses

bottom. The third section of the form (Pathologic Diagnosis) is intended for the histopathologic description and list of anatomic findings. The latter are customarily numbered in order of importance. The fourth and last section of the form has been arranged in columns corresponding to the four major groupings of the SNOP-Code plus the fifth "Phase" modifier. Thus, column "T" stands for Topography, "M" for Morphology, "E" for Etiology, and "F" for Function. The ascending numbers in the left column correspond to the numbered pathologic diagnoses elaborated upon in the section above. Where allocated space is insufficient for description, the information is continued on the reverse side of the form, or on additional attached pages.

Coded information supplied on the form may be readily entered into computer storage. Key-punch operators enter the pertinent information in the first section of the form, and then enter only the pathologic diagnoses from the fourth section of the form. If desired, it is also possible to enter, by code, the tissue lists in the right-hand column. The master or standard animal reference number always appears in the extreme upper left box of the first section. With this arrangement the pathology information is available for retrieval and program reduction by anyone for integration with his own data. Upon completion of the report, Xerox copies can be made and submitted to the individual laboratories for their immediate use. An example of such a completed form is shown in Figure 2.

The Requisition-Report form is printed on a relatively heavy grade of 8-1/2 by 11 inch paper (60 lb — "Moistrite" offset book paper, Mead Paper Corporation) which will withstand considerable abuse, and which is capable of Xerox copying on both sides without "show-through." This paper is also suitable for pencil and ink notations without bleeding. For convenience, the forms are bound into pads of 100 sheets each with three-hole punches on the extreme left margin for insertion into standard three-ring notebooks. Printing costs are modest.

Printed anatomic drawings of soft tissues and the skeletal system accompanying the Requisition-Report form enhance accuracy of gross

PATHOLOGY

ANIMAL / VET. MED. NUMBER	EXPERIMENT CODE NUMBER	PATH. SERIES INDEX NUMBER	PATHOLOGY ASC. NUMBER	SPECIES: Rat	
642625	135-58	3	S-67-A	AGE: 290	SEX: M

DATE YEAR	DATE DAY OF YEAR	ORIGINATING LABORATORY	ISOTOPE	AFTER DAYS EXPOSURE		SACRIFICE SERIES		DIED
65	98	RB	DOSE	253		LONGEVITY SERIES	x	SACRIF.

EXAMINER'S FINDINGS AND COMMENTS:

Animal sacrificed in dying state; body temperature depressed; secretions around eyes; labored breathing. Red cell count 4.5 million; hematocrit 28.3; buffy coat 14.7; hemoglobin 11.2 gm; MCV 62.6; MCHC 39.5; MCH 24.7. Leukocyte count 564,000, nearly all granulocytic series. Examination showed a typical picture of chloroma leukemia. All lymph nodes are markedly enlarged and of deep olive green coloration. Cervical, thoracic, abdominal, and pelvic lymph nodes share in this enlargement. The spleen is markedly enlarged, weighing 7 gm and measuring 74 mm in length and 24 mm in maximum width. Liver is of usual shape but enlarged and weighs 21.5 gm. (USE REVERSE SIDE FOR ADDITIONAL SPACE)

PATHOLOGY DIAGNOSIS:

1. Leukemia, chronic granulocytic, Sr-isotope, with widespread infiltrates in tissues, sparing brain, testes, salivary gland. Max. infiltrates in spleen, L.N., bone marrow, kidneys, liver, lungs, and prostate; patchy small infiltrates in G.I.T., retina, thyroid. 4/5 of pituitary replaced; basilar meninges involved. Chloroma.
2. Lymphadenopathy.
3. Splenomegaly.
4. Acute suppurative, confluent, lobular pneumonia; suppurative tracheobronchitis.
5. Focal marrow necrosis.
6. Focal glomerulo-sclerosis.
7. Erythroid metaplasia in liver, kidney, spleen.

SNOP CODE	T	M	E	F	P
1	0500	9867	5271		
2	0800	7200			
3	0800	7200			
4	2800	4121			0205
5	0600	5401	5271		
6	7100	5331			0103
7	0600	7783			
8					
9					
10					

x	SKIN
	BREAST
x	THYMUS
x	LYMPH NODES
x	SPLEEN
x	BLOOD
x	MARROW
x	BONE
	MUSCLE
	ADIPOSE
x	LARYNX-TRACHEA
	BRONCHUS
x	LUNG
x	HEART
	BLOOD VESSEL
	TEETH
	TONGUE
x	SALIVARY GLD.
	TONSILS
x	ESOPHAGUS
x	STOMACH
x	DUODENUM
x	ILEUM
x	COLON
x	LIVER
	GALL BLADDER
x	PANCREAS
x	KIDNEY
x	URETER
x	URINARY BLAD.
x	URETHRA
x	PROSTATE
x	SEMINAL VES.
x	TESTES
	VAGINA
	UTERUS
	FALLOPIAN T.
	OVARY
	PLACENTA
	FETUS
x	PITUITARY
x	ADRENAL
x	THYROID
x	PARATHYROID
	PARAGANGLIA
x	BRAIN
	NERVE
	EYE-ORBIT
	EAR-MASTOID
	SINUSES

dissection observations. Sketches and specifications of lesions are made directly on the drawing; examples of the form for rats are shown in Figures 3 and 4.

The forms have been well received by the participating laboratories and have proved to be a convenient method of maintaining and recording data and to be a distinct convenience to the pathologist in maintaining information in an orderly form as he reviews the histologic material.

FIGURE 3

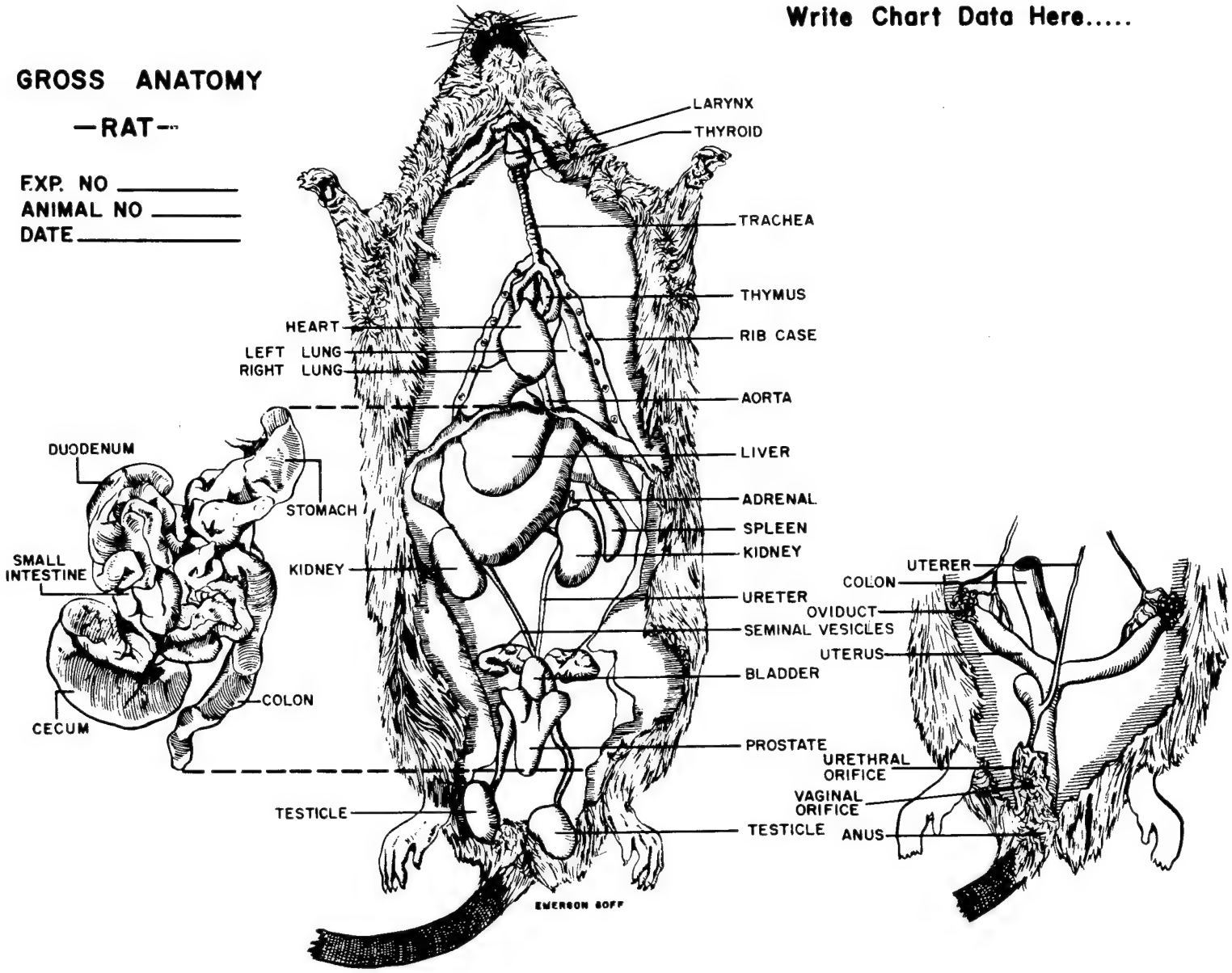
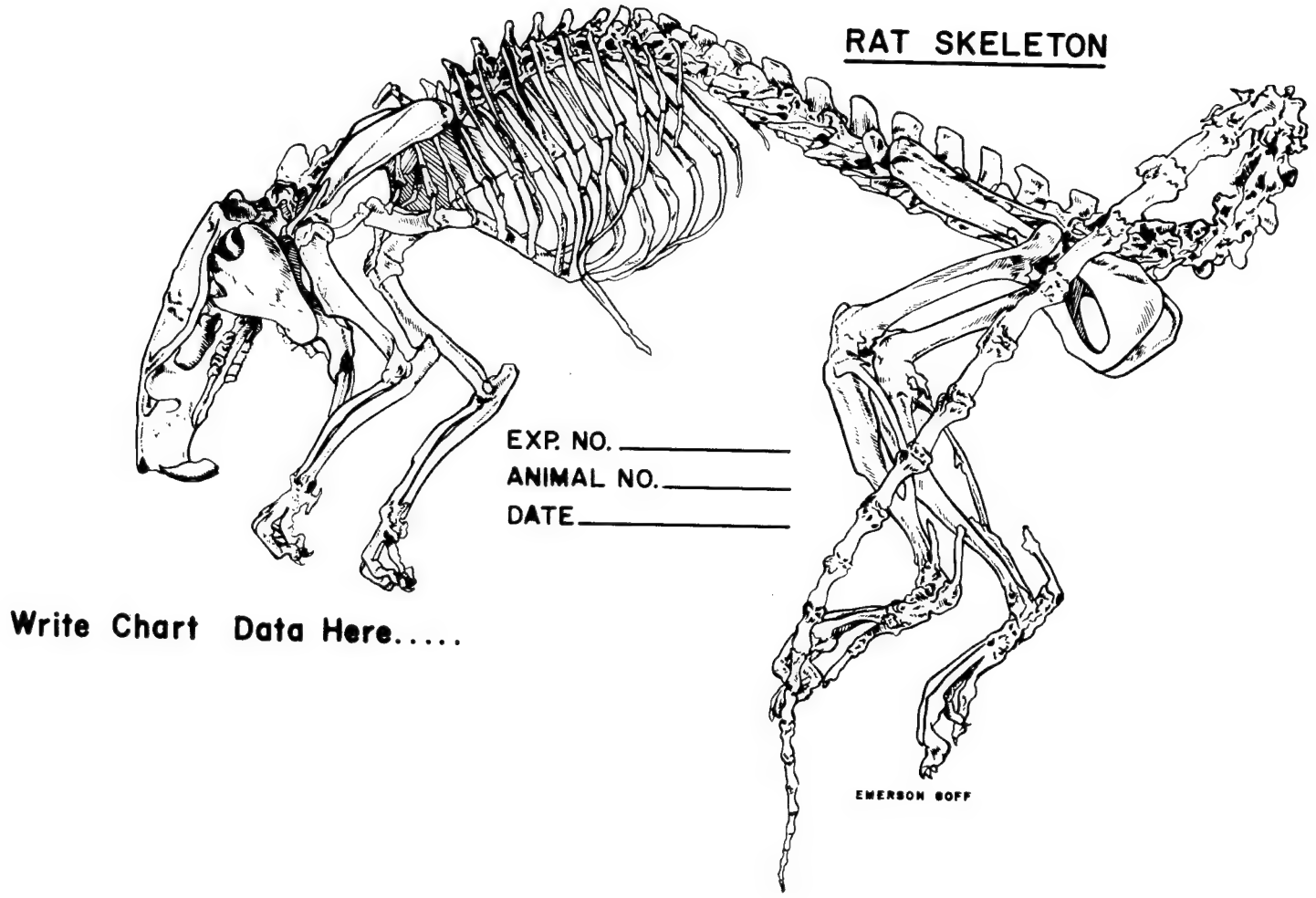

Write Chart Data Here.....
GROSS ANATOMY
—RAT—
EXP. NO _____
ANIMAL NO _____
DATE _____
LARYNX
THYROID
TRACHEA
THYMUS
RIB CASE
HEART
LEFT LUNG
RIGHT LUNG
AORTA
LIVER
ADRENAL
SPLEEN
KIDNEY
URETER
SEMINAL VESICLES
BLADDER
PROSTATE
TESTICLE
TESTICLE
DUODENUM
STOMACH
SMALL INTESTINE
KIDNEY
COLON
CECUM
UTERER
COLON
OVIDUCT
UTERUS
URETHRAL ORIFICE
VAGINAL ORIFICE
ANUS
EMERSON GOFF

FIGURE 4


RAT SKELETON
EXP. NO. _____
ANIMAL NO. _____
DATE _____
Write Chart Data Here.....
EMERSON GOFF

URANYL NITRATE-MONTMORILLONITE CLAY EXCHANGE

by

S. Posner and J. Bennick

INTRODUCTION

The successful ion exchange of certain fission products (Ce-141, Sr-90, Cs-137, Ba-La-140, Ru-106 and Y-91) to prepare insoluble aerosols prompted an investigation and evaluation of the possibility of exchanging uranyl ions with clay particles. It was thought that adsorption onto spheroidized clay particles to encapsulate the uranyl ion was practical. Then it was deemed feasible to irradiate this material to produce a mixed fission product aerosol. This possibility of using irradiated uranyl ion and clay was more attractive for the following reasons:

1. It eliminated the necessity of burning irradiated uranium metal to prepare mixed fission product aerosols.
2. It eliminated redesigning existing equipment for aerosol production in the animal exposure units.
3. It remained with a common carrier for insoluble aerosols.

EXPERIMENTAL WORK

Early laboratory attempts using natural uranyl nitrate subsequently irradiated were very encouraging. These initial tests indicated that:

1. Uranyl ion would exchange with the clay.
2. Heat required for encapsulation did not effect uranyl ion retention in the clay particles.
3. Irradiation of the clay beads produced the expected fission products for the exposure time in the Omega West Reactor*.

*Omega West Reactor at Los Alamos, New Mexico

Because of the favorable results it was decided to prepare some samples using enriched uranyl nitrate.

EXPERIMENTAL PROCEDURES

A 1.0% by weight solution of uranyl nitrate (45% enriched) was exchanged with 12.5 mgs. of 0.05 μ clay particles. After 24 hours the exchange was terminated, the clay particles were encapsulated, washed twice, dried thoroughly, then prepared for irradiation at Omega West.

Irradiation time for this experiment was limited to 12 hours since the exact amount of uranyl clay exchange was unknown. Results of this test will be used to plan further experiments. After irradiation the material was evaluated by two different methods, gamma-ray spectroscopy and alpha particle activity. The amount of uranyl ion exchange with the clay was calculated by determining the La-140 and subsequent determination of Ba-140. Once the amount of Ba-140 is determined, the original amount of uranyl ion adsorbed by the clay particles can be approximated (Appendix I).

This preliminary experiment indicated an uptake of uranyl ion by clay of 26%.

FUTURE EXPERIMENTS

One limitation of this procedure is the amount of U-235 that may be irradiated in the Omega West Reactor (50 mgs. U-235 per sample). Initial contacts have been established at the SERF reactor (Sandia Corporation) which can handle larger quantities of enriched uranyl ion clay particles. Future studies will include evaluation of particle size versus uptake of uranyl ion, evaluation of the amount of fission product buildup for several time intervals in the reactor and finally, pilot runs of the resultant aerosols in the animal exposure units.

APPENDIX I

CALCULATION OF URANYL ION UPTAKE IN CLAY

1. La-140 1.6 mev γ-activity as of 0800, 9/4/64 is equivalent to 1.01 μc La-140.
2. Total La-140 activity is 1.07 μc at t = 0.
3. 6.3% of all fissions produced a Ba-140 atom which decays with a half-life of 12.8 days to La-140 (half-life 1.675 days).

If A_{B_o} is the Ba-140 activity at t = 0, then the La-140 activity at t is:

$$A_L = \frac{\lambda_L A_{B_o}}{\lambda_L - \lambda_B} \left(e^{-\lambda Bt} - e^{-\lambda Lt}\right)$$

$$\lambda_L = \frac{.693}{1.675} \text{ days}^{-1} = .414 \text{ days}^{-1}$$

$$\lambda_B = \frac{.693}{12.8} \text{ days}^{-1} = .054 \text{ days}^{-1}$$

$$A_L = 1.149 A_{B_o} \left(e^{-.054t} - e^{-.414A}\right)$$

For t = 14 days (after irradiation)

$$A_L = 1.149 A_{B_o} (.47 - .003) = .539 A_{B_o}$$

$$A_{B_o} = 1.99 \mu c$$

4. Let w = number of mgms. of uranyl ion in the irradiated sample.
Let f = number of fissions/hr/gram of natural uranium.
Then the activity of Ba-140 formed in 12 hours of irradiation is:

$$A_{B_o} = 1.05 \times 10^{-6} wf(-e^{-.5 \times .054}) \text{ d/m}$$

$$= 4.73 \times 10^{-13} \times .027 wf\mu c$$

$$= 1.274 \times 10^{-14} wf\mu c$$

For natural uranium, $f = 6.06 \times 10^{13}$ gm.$^{-1}$ hr.$^{-1}$ and:

$$A_{B_o} = 0.772 w\mu c$$

$$w = A_{B_o} / .0772 = 1.99/0.772 = 2.58 \text{ mgms. uranyl ion}$$

Weight of clay was 12.5 mgms. indicating an uptake of uranyl ion of 20.6% by weight.

A BACTERIOLOGICAL SURVEY OF COMMERCIALLY AVAILABLE LABORATORY RATS

by

J. H. Sherrod and E. Kinter

This preliminary survey was initiated in an attempt to find a commercial source of rats with a minimal incidence of chronic respiratory disease (CRD). A bacteriological survey of the lungs, upper respiratory tract and middle ear of rats from various suppliers was conducted. Several organisms found in respiratory tracts and middle ears of rats have been reported, notably work by Nelson[1] in which virus and mycoplasmas (PPLO) were incriminated. In his earlier work[2] Streptobacillus moniliformis was also discussed as an etiological agent of CRD.

METHODS

Animals were randomly selected from each shipment and bacteriological samples were taken initially from the lung, trachea, pharynx and middle ear. Organisms were inoculated on blood agar plates, Trypticase Soy Broth (Baltimore Biological Laboratory, Baltimore, Maryland) and PPLO media, then picked to pure culture. Routine identification was made.

RESULTS

Listed in order of predominance are the organisms isolated from shipments from eight different suppliers:

1. Pasteurella pneumotropica (confirmed by Communicable Disease Center, Atlanta, Georgia (CDC).
2. An unidentified small gram-positive bacillus having culture characteristics suggesting Erysipelothrix spp.
3. Pseudomonas sp.

4. Pasteurella gallinarium (confirmed by CDC).
5. Diplococcus Pneumoniae
6. Clostidium perfringens
7. Flavobacterium sp.

This laboratory has been unable to isolate PPLO from these commercially available animals. It has been noted that the gross appearance of purulent material in the middle ear and macroscopic lesions in the lungs were not consistent with isolation of the organisms. In several animals showing no gross lesions, organisms were isolated from these organs. Isolation of bacteria, notably Pasteurella pneumotropica, was made from more of the middle ear samples than from lungs of the same animals. The trachea and pharynx cultures were discontinued after lack of discovering significant differences in the isolates from those found in the lung.

It is emphasized that possibility of environmental exposure and infection during shipment cannot be ruled out. Infection of these animals in transit, from human sources, is a definite possibility and during the next year it is planned to expand this study.

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LABORATORY RODENT CARE PROGRAM

by

H. C. Redman, J. H. Sherrod, F. A. Malone and E. Kinter

INTRODUCTION

The purpose of this program is to provide the best professional care available for the experimental laboratory rodent colony that is being utilized to study the biological effects of the inhalation of mixed fission products. Procedures for the basic care and management of these laboratory rodents has been published elsewhere.[1] These include the routine daily care and feeding, observation, medical care, sample collection and inventory of the colony. During the time period 1 July 1964 to 30 June 1965, 3454 laboratory rodents have been processed through these standard isolation procedures. They have been used for longevity, effects, distribution and excretion, and deposition studies. As of 30 June 1965, the colony contained 930 experimental animals and 500 within the breeding and stock colony.

All rodents assigned to the lifespan colony are weighed and examined individually every two weeks. At this time there are 550 animals in lifespan studies, and this number has increased throughout the year.

Routine fecal examinations for Salmonella sp. within the commercially supplied laboratory rodents are conducted upon arrival at the facility. Specimens are taken from each cage which houses either one guinea pig, two rats or six mice. There have been 1897 examinations during the year with no positive isolations. This program for surveying the animals has been conducted to prevent the spread of and/or increase in the amount of Pseudomonas within the colony. There was a noticeable decline in the number of positive cultures during the last three months of the year. Periodic examinations are being made of equipment utilized within the laboratory rodent colony as a check on the degree of sanitation being obtained

by the washing procedures. The method utilized for routine Pseudomonas cultures follows.

Pseudomonas Culture:

1. The inside of a drinking tube or inside of the lid of a water bottle is swabbed using a sterile swab. (Q-tip type or applicator stick.)
2. The swab is placed in Wensinck media (glycerol broth).[2]

Glycerol	1% (w/v)
Difco Proteose Peptone	1.5%
K_2HPO_4	0.04%
$MgSO_4 \cdot 7H_2O$	2.0%
$FeSO_4$	0.001%

The pH is adjusted to 7.5 before sterilization at 120° C for 15 minutes. The medium is filtered and sterilized again at 110° C for 10 minutes.

3. Examination is made for blue-green pigment in 72 hours. (If no color is apparent in the tube a chloroform extraction is made prior to declaring it a negative culture.) Blue-green pigment indicates Pseudomonas.

REFERENCES

1. Redman, H. C. and J. H. Sherrod, "Management Procedures for Animal Colonies Serving the Fission Product Inhalation Program," Lovelace Foundation Report, LF-24, 1965.

2. Wensinck, F., D. W. Van Bekkum and H. Renaud, "The Prevention of Pseudomonas Infection in Irradiated Mice and Rats," Rad. Res. 7: 491-499, 1957.

BIBLIOGRAPHY

1. Flynn, R., "The Diagnosis of Pseudomonas aerudinosa Infection in Mice," Lab. Animal Care 13: 126-129, 1963.

USE OF NITROFURAZOLIDONE TO CONTROL *PASTEURELLA PNEUMOTROPICA* INFECTION IN RATS

by

J. H. Sherrod and E. Kinter

This study was initiated to evaluate the use of nitrofurazolidone (Furoxone by Eaton Laboratories, Norwich, New York) in rats. The use of this drug in commercial guinea pig feed (Cali Pellets by R. Cali and Brother, Cupertino, California) suggested the possibility of using it as a means of controlling *Pasteurella pneumotropica* in rats.

To test this, 0.0055 per cent was added to the drinking water of a group of 24 rats infected with *Pasteurella pneumotropica*. A control group of 24 unmedicated rats infected with this organism was used for comparison. Two experimental and two control animals were sacrificed at one-week intervals. Culture samples were taken of the lung, middle ear, intestine, and cecum.

A change in intestinal flora was observed in the experimental group beginning with the first week. No *Pasteurella pneumotropica* were isolated after that time, however, *Diplococcus pneumoniae* was isolated throughout the entire study. Although this method of treatment reduced the incidence of *Pasteurella pneumotropica* markedly, it was ineffective in reducing the incidence of chronic respiratory disease. In particular, the gram-positive organisms associated with this condition showed no reduction.

DOG CARE PROGRAM

by

H. C. Redman, J. H. Sherrod, W. F. Jordan and N. Pollock

The basic care and management of the dogs in this colony has been published elsewhere.[1] This includes routine care and feeding, daily observation, medical care and assistance in collection of biological samples for other Departments. The colony inventory on July 1, 1965 was 544 beagles; 56 on long term experiments, 70 for breeding, 356 less than one year of age and 62 adults on short term studies. An annual examination is performed on each dog at 12 months (± 2 weeks) of age and thereafter on its birthdate. It consists of a physical examination (Tables 1-4) and a standard set of diagnostic X-rays (Tables 5, 6). The forms shown in Tables 1-4 were designed for both ease of use during the physical examination and simplicity of input to the Foundation computer for storage, future retrieval and analysis. The result of each segment of the examination is circled on an entry made in the allotted space. Entries may be numeric values, narrative, or coded data. Code systems are presently used for location of abnormalities (Figures 1-5) and dental examination (Figure 6). The 56 physical examinations on dogs entering the lifespan studies have shown no important abnormalities.

In addition to the annual physical examination, clinical tests are performed throughout the year to assess the general health and care of the breeding and puppy colonies. These examinations are performed on a routine schedule; details and results to date are presented below.

URINALYSIS

Procedure:

1. Sample Collection:

The sample is collected by compression, catheterization or from the

TABLE 1

PHYSICAL EXAMINATION

Form V-22 1-4

A. TATTOO NO. _____

B. TYPE
1. Semi-annual
2. Annual
3. Special

C. SEX M F

D. DATE - yr. _____ day _____

E. HOUR _____ A.M. _____ P.M.

F. WEIGHT _____ g.

G. BODY TEMPERATURE _____ °F

H. PULSE RATE _____

I. RESPIRATION
1. Rate/minute _____
2. Depth
a. decreased
b. normal
c. increased
3. Character
a. normal
b. abnormal
1) description _____

J. CONFORMATION
1. Normal
2. Abnormal
a. description _____

K. GENERAL CONDITION
1. Comatose
2. Extremely poor
3. Very poor
4. Poor
5. Poor to fair
6. Fair
7. Fair to good
8. Good
9. Good to excellent
10. Excellent

L. TEMPERAMENT
1. Normal
2. Abnormal
a. nervous (slight)
b. nervous (moderate)
c. nervous (very, but cooperative)
d. nervous (very, uncooperative)
e. aggressive
f. vicious
g. docile
h. lethargic
i. other _____

M. OBESITY
1. Thin, very
2. Thin, moderate
3. Thin, slight
4. Normal
5. Obese, slight
6. Obese, moderate
7. Obese, very

N. MEASUREMENTS
1. Length _____ cm
2. Height _____ cm
3. Width (Thorax) _____ cm
4. Depth _____ cm

O. HAIR LENGTH
1. Head _____ cm
2. Thorax _____ cm
3. Neck _____ cm
4. Axilla _____ cm
5. Abdomen _____ cm
6. Back _____ cm
7. Front legs _____ cm
8. Hind Legs _____ cm
9. Tail _____ cm

P. HAIR TEXTURE
1. Normal
2. Abnormal
a. very fine
b. fine
c. coarse
d. very coarse
e. other _____

Q. HAIR CONDITION
1. Normal
2. Abnormal
a. oily
b. dry
c. shedding
d. other _____

R. GREYING
1. Absent
2. Generalized
3. Head
4. Chin
5. Muzzle
6. Neck
7. Thorax
8. Axilla
9. Abdomen
10. Back
11. Legs, front
12. Legs, hind
13. Tail

S. SKIN
1. Normal
2. Abnormal
a. Nonparasitic Dermatitis
1) acute inflammation
2) chronic inflammation
3) pyogenic dermatitis
4) seborrheic dermatitis
5) allergic dermatitis
6) eczematous dermatitis
7) manifestation of systemic disease
8) alopecia
9) color abnormality (hair)
10) hyperpigmentation
11) depigmentation
12) hyperemia
13) jaundice
14) depilitation
15) broken hair
16) sebum
17) skin adema
18) sub-cutaneous emphasema
19) hypertropy
20) abnormal odor
21) dry
22) macules (spots)
23) papules (pimples)
24) nodules
25) urtica (wheal)
26) vesicle
27) pustules
28) excoriations
29) rhayades
30) ulcers
31) scabs (crusts)
32) scales (squamae)
b. Parasitic dermatitis
1) acariasis
a) demodex
b) sarcoptes
2) dermatolycoses
a) microsporum
b) trichophyton
3) other _____
c. Location _____
d. Severity
1) very mild
2) mild
3) severe
4) very severe

T. WOUNDS
1. Absent
2. Present
a. location _____
b. condition _____

U. NEOPLASIA
1. Absent
2. Present
a. location _____
b. characteristic _____

V. NODES
1. Right madibular
a. normal
b. abnormal
1) characteristic _____
2. Left madibular
a. normal
b. abnormal
1) characteristic _____
3. Right parotid
a. normal
b. abnormal
1) characteristic _____
4. Left parotid
a. normal
b. abnormal
1) characteristic _____
5. Right prescapular
a. normal
b. abnormal
1) characteristic _____
6. Left prescapular
a. normal
b. abnormal
1) characteristic _____
7. Right axillary
a. normal
b. abnormal
1) characteristic _____
8. Left axillary
a. normal
b. abnormal
1) characteristic _____
9. Right superficial inguinal
a. normal
b. abnormal
1) characteristic _____

TABLE 2

10. Left superficial inguinal
    a. normal
    b. abnormal
        1) characteristic_____
11. Right popliteal
    a. normal
    b. abnormal
        1) characteristic_____
12. Left popliteal
    a. normal
    b. abnormal
        1) characteristic_____
13. Other
    a. normal
    b. abnormal
        1) characteristic_____

W. UDDER CONDITION
1. Development
    a. infantile
    b. slight
    c. moderate
    d. complete
    e. extra large

X. PERINEAL AREA
1. Normal
2. Abnormal
    a. enlarged anal pouches
    b. perineal irritation
    c. perineal hernia
        1) unilateral
            a) right
            b) left
        2) bilateral
    d. inguinal hernia
        1) unilateral
            a) right
            b) left
        2) bilateral
    e. other_____

Y. RECTUM
1. Normal
2. Abnormal
    a. condition_____

Z. TAIL
1. Normal
2. Abnormal
    a. condition_____

AA. FEET
1. Normal
2. Abnormal
    a. condition_____

BB. NAILS
1. Normal
2. Abnormal
    a. wear
        1) slight
        2) heavy
    b. torn
    c. missing
    d. other_____

CC. VULVA
1. Anestrus (normal)
2. Proestrus (slight enlargement)
3. Proestrus (enlarged)
4. Proestrus (enlarged, hemorrhage)
5. Estrus (enlarged, hard, no discharge)
6. Metestrum (enlarged, soft)
7. Abnormal vaginal discharge
8. Post-gestational discharge
9. Other_____

DD. TESTICLES
1. Normal
2. Abnormal
    a. retained
        1) right
        2) left
        3) both
    b. increased size
        1) right
        2) left
        3) both
    c. decreased size
        1) right
        2) left
        3) both
    d. orchitis
        1) right
        2) left
        3) both
    e. other_____

EE. PENIS
1. Normal
2. Abnormal
    a. phimosis
    b. paraphimosis
    c. balantis
    d. balanoposthitis
    e. infantile
    f. other_____

FF. OPHTHALMOLOGIC
1. Orbit
    a. normal
    b. abnormal
        1) characteristic_____
2. Eyeball
    a. normal
    b. abnormal
        1) exophthalmos
        2) enophthalmos
        3) hydrophthalmos
        4) other_____
3. Conjunctiva
    a. normal
    b. abnormal
        1) mild inflammation
        2) moderate inflammation
        3) severe inflammation
        4) other_____
4. Ocular discharge
    a. absent
    b. present
        1) serus
        2) purulent
        3) other_____
5. Eyelids
    a. normal
    b. abnormal
        1) blepharitis
        2) skin lesion
        3) ptosis
        4) trichiasis
        5) distichiasis
        6) wounds
        7) scar
        8) entroplan
        9) ectropian

6. Membrana nicitains
    a. normal
    b. abnormal
        1) harderian gland inflammation
            a) acute
            b) chronic
        2) other_____
    c. removed
7. Lacrimal duct
    a. normal
    b. abnormal
        1) stenosis
        2) inflammation
        3) other_____
8. Cornea
    a. normal
    b. abnormal
        1) keratitis
        2) pigment
        3) ulcer
        4) foreign body
        5) other_____
9. Anterior chamber
    a. normal
    b. abnormal
        1) hypopian
        2) keratitic precipitates
        3) hyphema
        4) other_____
10. Iris
    a. normal
    b. abnormal
        1) iritis
        2) iridocyclitis
        3) coloboma
        4) anterior synechiae
        5) posterior synechiae
        6) other_____
11. Lens
    a. normal
    b. abnormal
        1) iridodoneses
        2) luxation (posterior)
        3) luxation (anterior)
        4) cataract
        5) other_____
12. Retina
    a. normal
    b. abnormal
        1) tapetum lucidium anomaly
        2) tapetum nigrum anomaly
        3) other_____
13. Reaction of Pupils
    a. normal
    b. abnormal
        1) condition
14. Degree of vision

	Rt.	Lft.
a. normal	1)_____	2)_____
b. impaired	1)_____	2)_____
c. blind	1)_____	2)_____

GG. BUCCAL CAVITY
1. Lip upper
    a. normal
    b. abnormal
        1) lesion

TABLE 3

TATTOO NO. _____ Form V-22 3-4

2. Lip lower
   a. normal
   b. abnormal
      1) lesion
3. Tongue
   a. normal
   b. abnormal
      1) lesion
4. Cheek
   a. normal
   b. abnormal
      1) lesion
5. Hard palate
   a. normal
   b. abnormal
      1) lesion
6. Soft palate
   a. normal
   b. abnormal
      1) lesion
7. Pharynx
   a. normal
   b. abnormal
      1) lesion
8. Tonsillar tissue
   a. normal
   b. abnormal
      1) lesion

HH. GUMS
1. Normal
2. Gingivitis
   a. slight
   b. moderate
   c. marked
3. Receding
   a. slight
   b. moderate
   c. marked
4. Hyperplastic
   a. slight
   b. moderate
   c. marked

II. DENTITION
1. Normal
2. Tartar accumulation
   a. slight
   b. moderate
   c. marked
3. Periodontal disease
4. Absent
5. Broken
6. Other _____
7. Other _____
8. Other _____
9. Other _____

Tooth

1. _____	14. _____
2. _____	15. _____
3. _____	16. _____
4. _____	17. _____
5. _____	18. _____
6. _____	19. _____
7. _____	20. _____
8. _____	21. _____
9. _____	22. _____
10. _____	23. _____
11. _____	24. _____
12. _____	25. _____
13. _____	26. _____

Tooth

27. _____	35. _____
28. _____	36. _____
29. _____	37. _____
30. _____	38. _____
31. _____	39. _____
32. _____	40. _____
33. _____	41. _____
34. _____	42. _____

JJ. NOSE
1. Normal
2. Abnormal
   a. discharge
      1) serus
      2) mucus
      3) muco-purulent
      4) purulent
      5) epitaxis
   b. other _____

KK. AURICULAS Rt. Lft.
1. Normal a) _____ b) _____
2. Abnormal
   a. condition _____

LL. EXTERNAL ACOUSTIC MEATUS Rt. Lft.
1. Normal a) _____ b) _____
2. Abnormal
   a. otitis externa
      1) degress
         a) slight *a* _____ *b* _____
         b) severe *a* _____ *b* _____
      2) condition
         a) acute *a* _____ *b* _____
         b) chronic *a* _____ *b* _____
      3) etiology
   b. other _____

MM. AUDITION Rt. Lft.
1. Normal a) _____ b) _____
2. Impaired a) _____ b) _____
3. Deaf a) _____ b) _____
4. Other a) _____ b) _____

NN. THORAX
1. Percussion
   a. animal uncooperative
   b. normal
   c. abnormal
      1) posterior border displacement
         a) anterior
         b) posterior
      2) increased resonance
         a) generalized
         b) localized
            a location _____
      3) decreased resonance
         a) generalized
         b) localized
            a location _____
2. Auscultation
   a. animal uncooperative
   b. animal obese
   c. normal
   d. abnormal
      1) vesicular sounds
         a) increased
            a generalized
            b localized
               1) location _____
         b) decreased
            a generalized
            b localized
               1) location _____
      2) bronchial sounds
         a) increased
            a generalized
            b localized
               1) location _____
         b) decreased
            a generalized
            b localized
               1) location _____
      3) adventitious sounds
         a) dry rales
            a generalized
            b localized
               1) location _____
         b) moist rales
            a generalized
            b localized
               1) location _____
         c) crepitant rales
            a generalized
            b localized
               1) location _____
         d) pleural rales
            a generalized
            b localized
               1) location _____
         e) other
            a generalized
            b localized
               1) location _____

OO. CARDIOVASCULAR
1. Auscultation
   a. normal
   b. abnormal
      1) description _____
2. Palpation (precardial area)
   a. normal
   b. abnormal
      1) description _____
3. Femoral pulse (simultaneous femoral arteries)
   a. normal
   b. abnormal
      1) description _____

PP. ABDOMEN
1. Size
   a. normal
   b. abnormal
      1) enlarged
      2) other _____
2. Percussion
   a. normal
   b. abnormal
      1) increased resonance
         a) generalized
         b) localized
            (1) location _____
      2) decreased resonance
         a) generalized
         b) localized
            (1) location _____
3. Palpation
   a. animal uncooperative
   b. animal obese
   c. normal

TABLE 4

3. Palpation
d. abnormal
1) abdomenal sensitivity
a) generalized
b) localized
(1) location _____
2) foreign mass
a) location _____
b) description _____
3) other _____
4. Auscultation
a. normal
b. abnormal
1) hypomotility
2) hypermotility
3) other _____

QQ. NEUROLOGIC REFLEXES
1. Spinal reflex
a. flexar reflex
1) normal
2) abnormal
a) description _____
b. knee jerk
1) normal
2) abnormal
a) description _____
c. extensor thrust reflex
1) normal
2) abnormal
a) description _____
2. Attitudinal reflex
a. tonic neck reflex
1) dorsal head flexion
a) normal
b) abnormal
(1) location _____
2) ventral head flexion
a) normal
b) abnormal
(1) description _____
3) side head flexion
a) normal
b) abnormal
(1) description _____
4) tonic eye reflex
a) normal
b) abnormal
(1) description _____
b. supporting reactions
1) normal
2) abnormal
a) description _____
c. righting reaction
1) normal
2) abnormal
a) description _____
d. placing reaction
1) normal
2) abnormal
a) description _____
e. hopping reaction
1) normal
2) abnormal
a) description _____

RR. COMMENT

1 _____
2 _____
3 _____
4 _____
5 _____
6 _____
7 _____
8 _____
9 _____
10 _____
11 _____
12 _____
13 _____
14 _____
15 _____
16 _____
17 _____
18 _____
19 _____
20 _____
21 _____
22 _____
23 _____
24 _____

TABLE 5

STANDARD RADIOGRAPHIC SURVEY

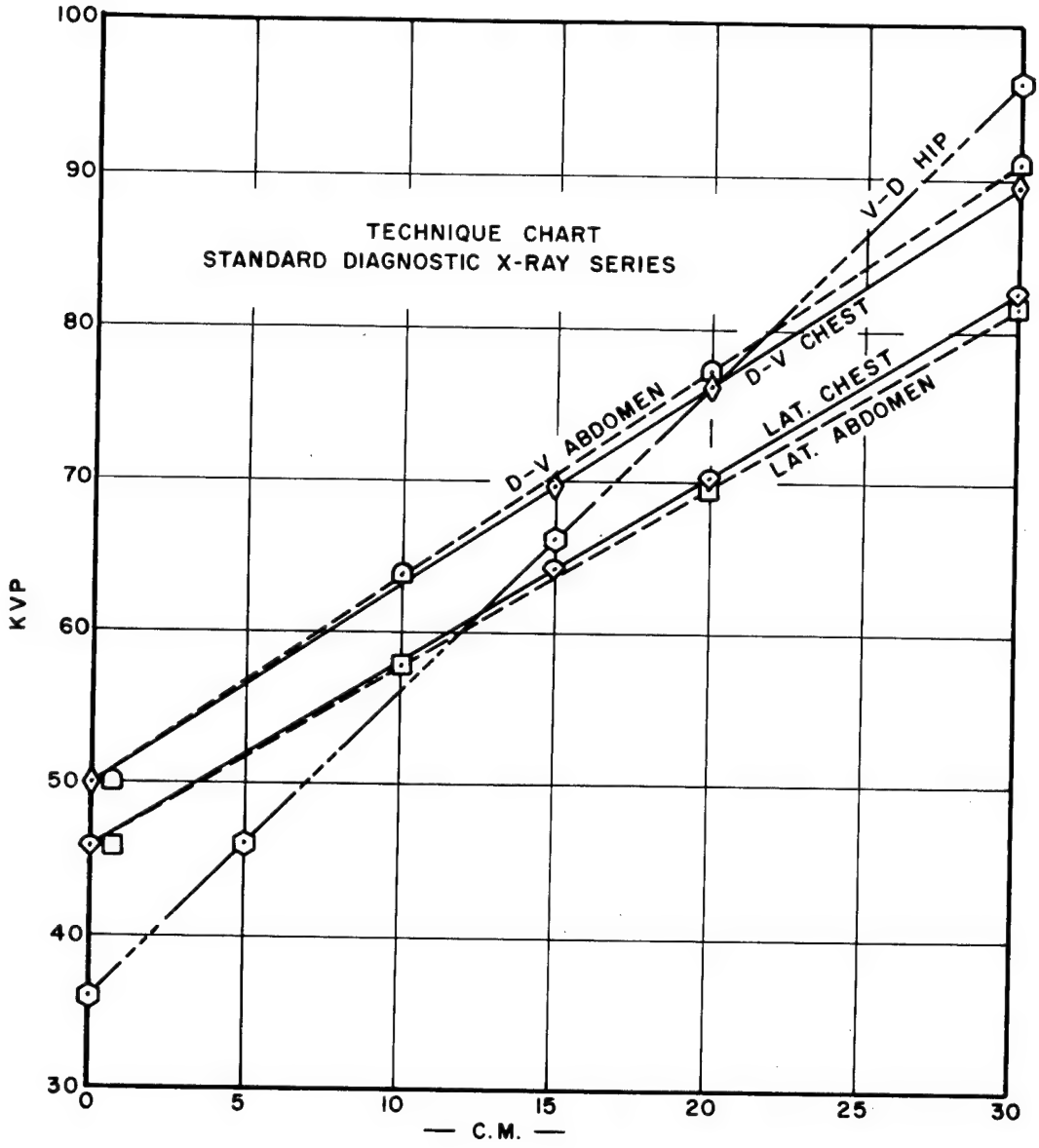
	Position	Region	Thick (Cm)	Dist (in)	KVP	MA	SEC
1.	D-V	Chest	*	40	*	200	1/15
2.	D-V	Abdomen	*	40	*	200	1/15
3.	Lat.	Abdomen	*	40	*	200	1/15
4.	Lat.	Chest	*	40	*	200	1/15
5.	V-D	Hip	*	40	*	200	2/15
6.	D-V	Skull	----	40	68	200	1/20
7.	Lat.	Skull	----	40	68	200	1/20
8.	R. Oblique	Skull	----	40	68	200	1/20
9.	L. Oblique	Skull	----	40	68	200	1/20
10.	Lat.	Front Legs	----	40	55	200	1/2
11.	Lat.	Rear Legs	----	40	58	200	1/2
12.	D-V	Front Legs	----	40	55	200	1/2

* Technique Chart utilized

1-9 Bucky utilized

10-12 Nonscreen film utilized

TABLE 6


TECHNIQUE CHART
STANDARD DIAGNOSTIC X-RAY SERIES
V-D HIP
D-V CHEST
D-V ABDOMEN
LAT. CHEST
LAT. ABDOMEN
KVP
100
90
80
70
60
50
40
30
0
5
10
15
20
25
30
— C.M. —

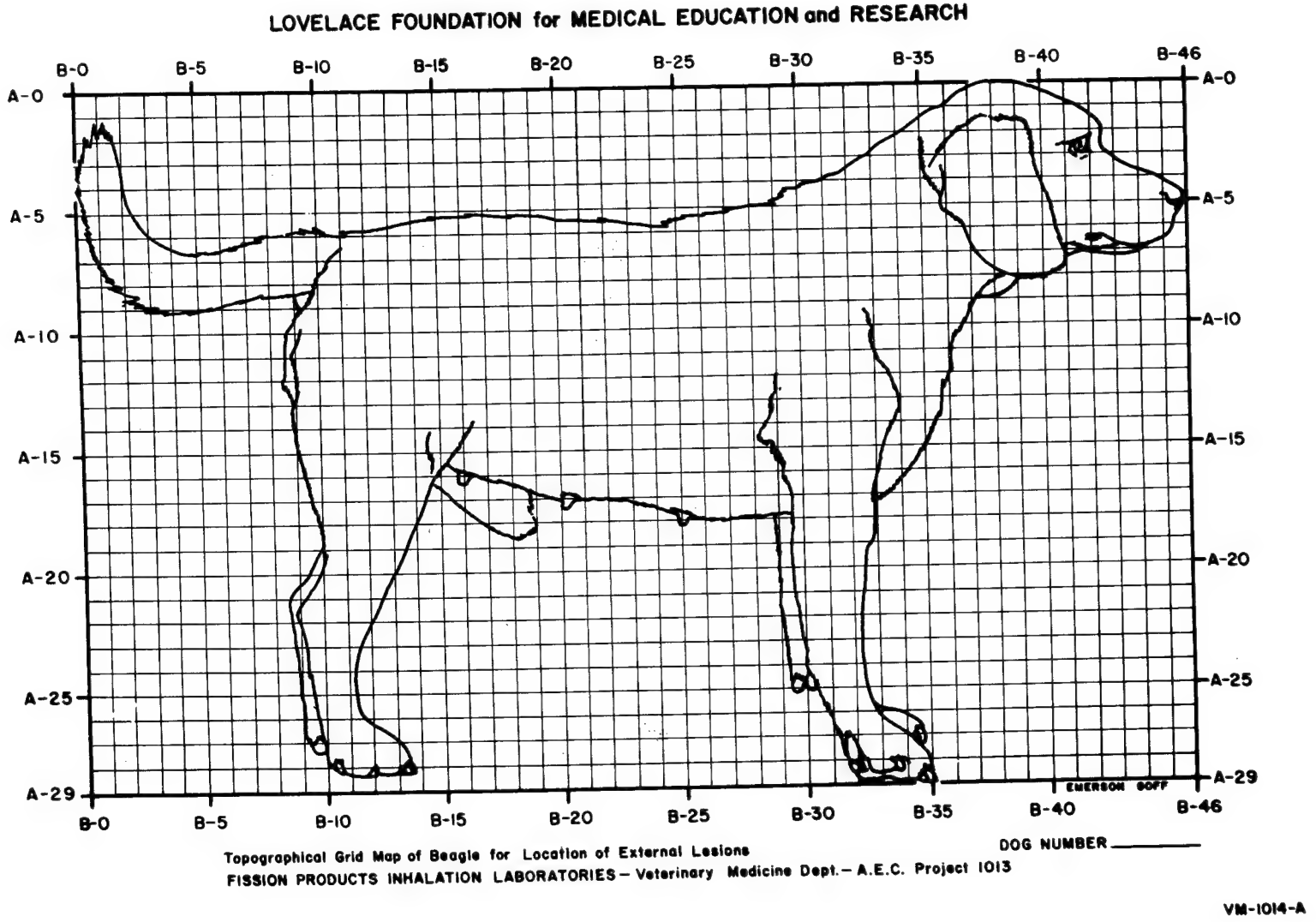

LOVELACE FOUNDATION for MEDICAL EDUCATION and RESEARCH
B-0
B-5
B-10
B-15
B-20
B-25
B-30
B-35
B-40
B-46
A-0
A-5
A-10
A-15
A-20
A-25
A-29
EMERSON GOFF
DOG NUMBER
Topographical Grid Map of Beagle for Location of External Lesions
FISSION PRODUCTS INHALATION LABORATORIES – Veterinary Medicine Dept. – A.E.C. Project 1013
VM-1014-A

FIGURE 1

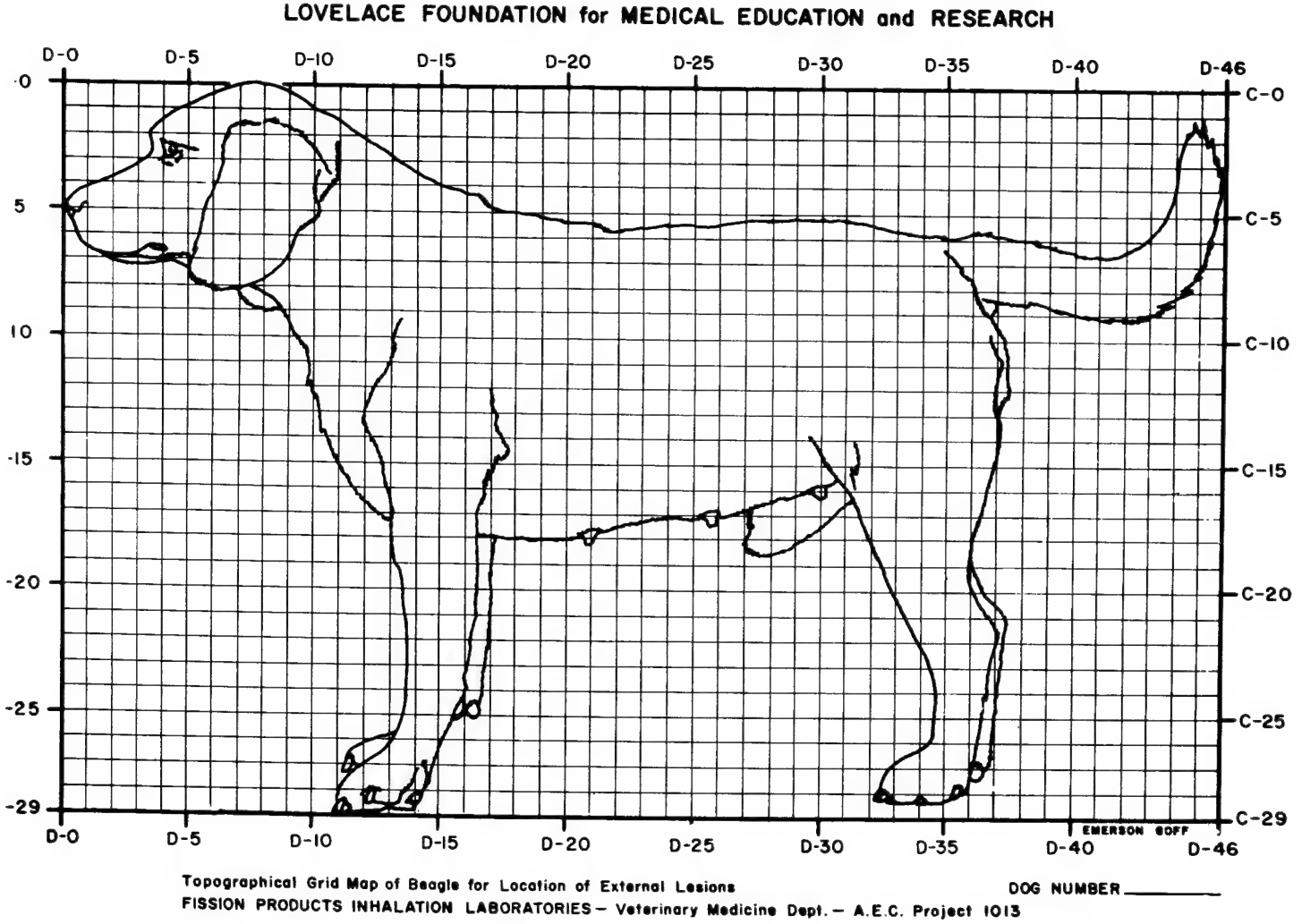


FIGURE 2

VM-1014-B

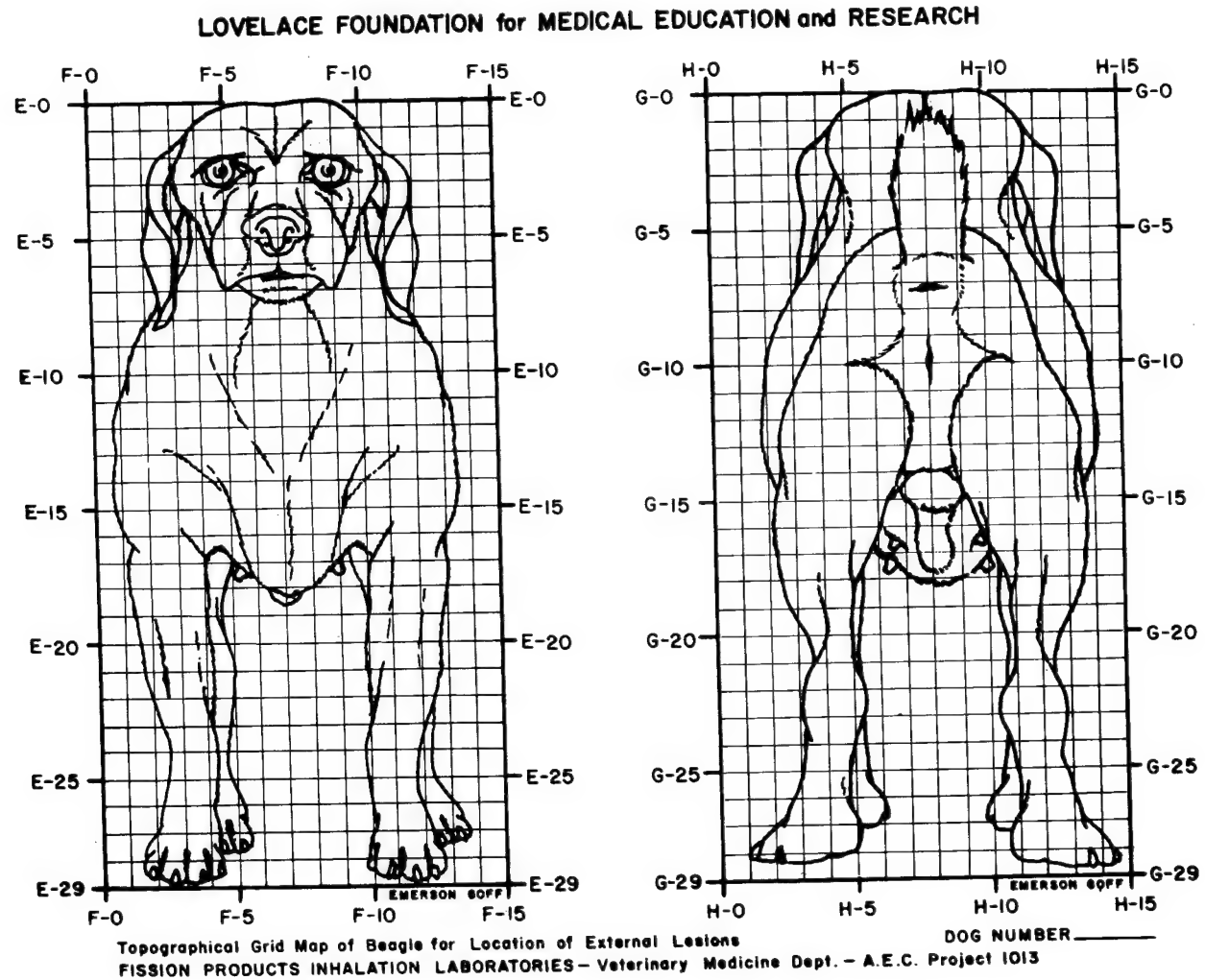


FIGURE 3

VM-1014-C

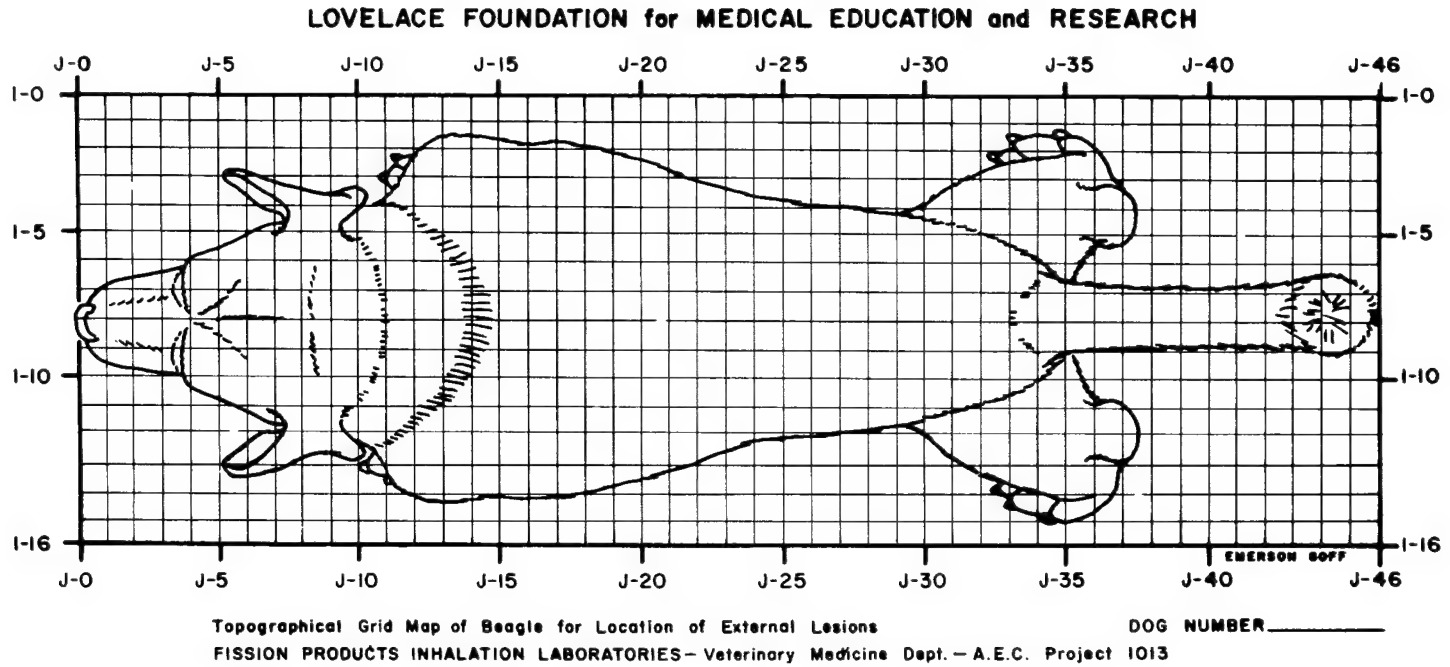
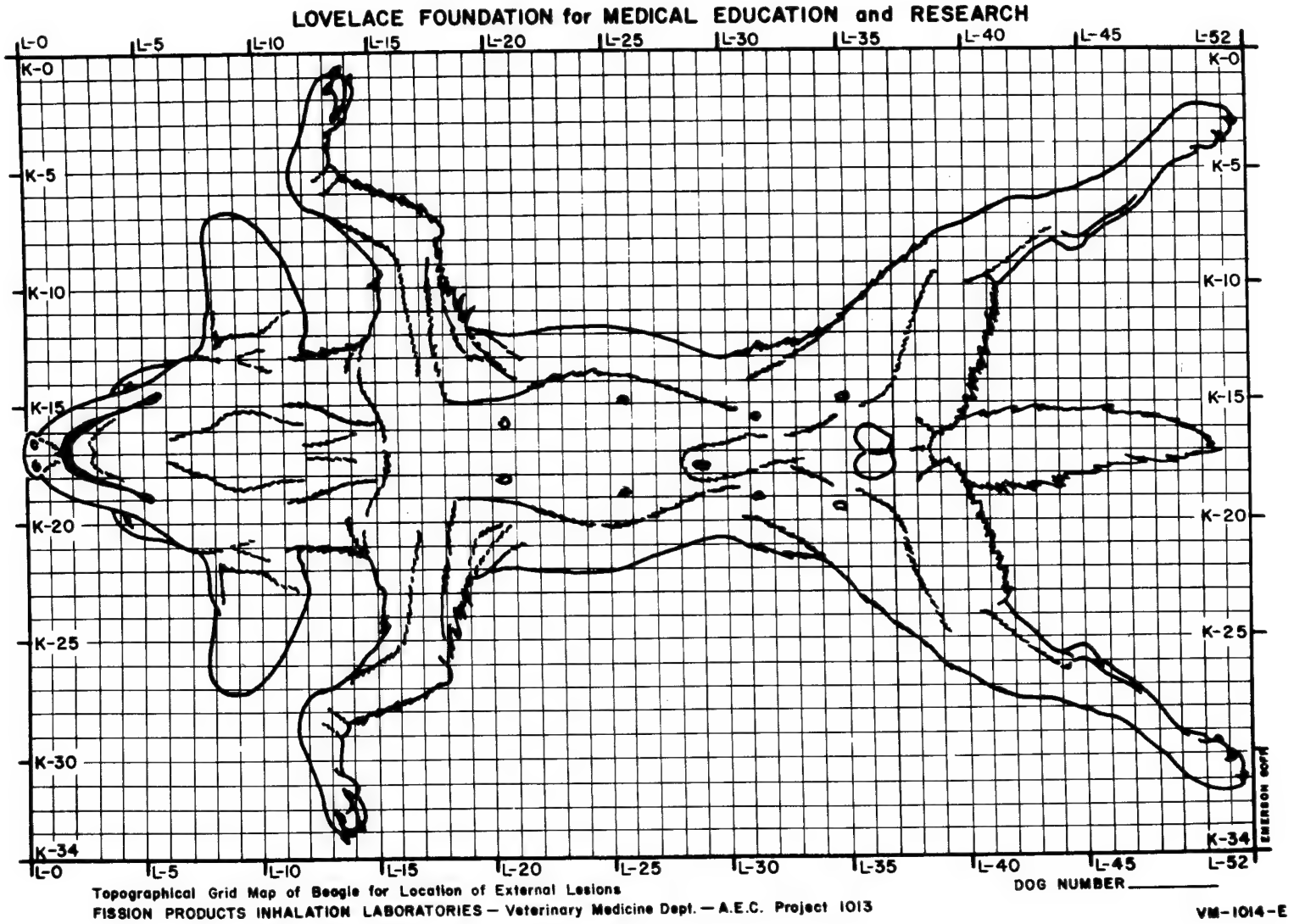


FIGURE 4

VM-1014-D



Topographical Grid Map of Beagle for Location of External Lesions
FISSION PRODUCTS INHALATION LABORATORIES — Veterinary Medicine Dept. — A.E.C. Project 1013

VM-1014-E

FIGURE 5

DENTITION OF BEAGLE

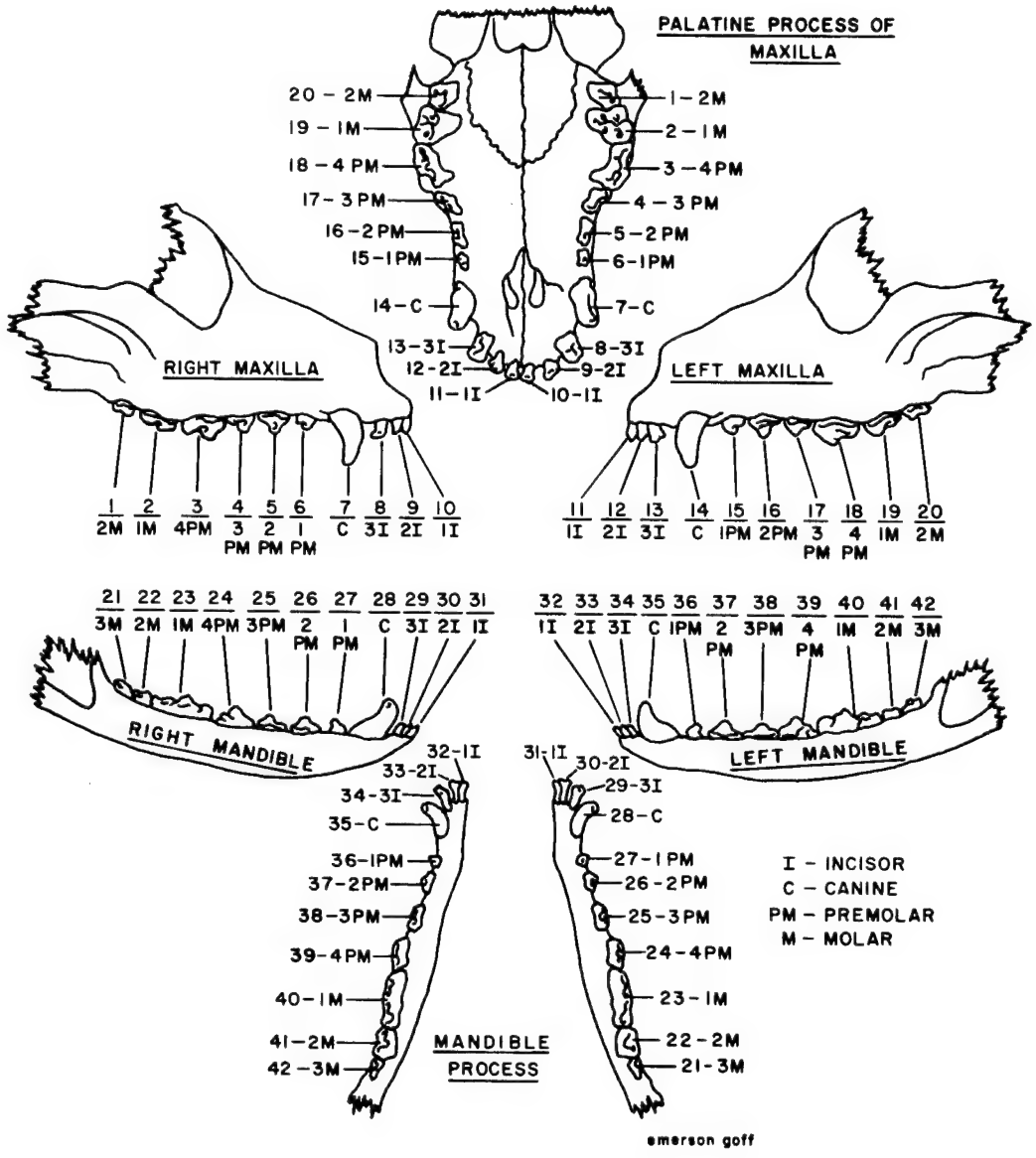


FIGURE 6

cage. The method of collection is recorded.

2. Gross Urinalysis:

   a. Specific gravity - the urine is measured in a Clay-Adams midgit urinometer and read to the closest 0.001 between 1.000 and 1.045.

   b. pH - a Combistix (Ames Company, Inc., Elkhart, Indiana) dipstick is used which reads to the nearest 0.5 between 5.0 and 9.0.

   c. Sugar - a Combistix dipstick is used with measurements determined from negative to 4+. All positive Combistix sugar tests are checked with Clinitest (Ames Company, Inc., Elkhart, Indiana) tablets.

   d. Albumin - a Combistix dipstick is used with measurements from negative to 4+. All positive Combistix albumins are checked with sulfasalicylic acid. Equal parts of urine and 3% sulfasalicylic acid are mixed and the turbidity is estimated from negative to 4+.

   e. Acetone - one drop of urine is placed on an Acetest tablet (Ames Company, Inc., Elkhart, Indiana) and the results read from negative to 4+.

   f. Bilirubin - two ml of urine are overlaid on 2 ml of fuming nitric acid (Gmeling's test). A multicolored ring at the junction of acid and urine indicates the presence of bile.

   g. Occult blood - a Guiac-tab (Cambridge Chemical Products, Dearborn, Michigan) is placed on filter paper. Two drops of urine are placed on tablet followed by 2 drops of glacial acetic acid and 2 drops of 3% hydrogen peroxide. Green to blue color on the filter paper indicates the presence of occult blood.

3. Microscopic Examination:

   Urine is placed in 12 ml tubes and centrifuged 5 minutes at 1500 rpm. The supernatant is discarded, the residue mixed and a drop examined under a cover slip for formed elements.

   a. Low power - casts and epithelial cells are looked for and it is determined if casts are hyaline, coarsely or finely granular, WBC, RBC or other, and the number of casts per low power field

is reported.

b. High power - RBC, WBC, bacteria, crystals and kidney cells are looked for and the number or amount per high power field is reported.

NOTE: If color or character of urine appears abnormal it is noted on the report; awareness should be made of fecal contamination.

PROGRESS REPORT

These procedures have been used on the 56 beagles in the long term experimental study and on the 70 adult dogs received for breeding and D & E studies. The data obtained to date on these dogs appeared normal; routine urinalyses will be done on all exposed dogs.

FECAL EXAMINATIONS

Procedure:

1. Reagent: Zinc Sulfate ($ZnSO_4 \cdot 7H_2O$ solution) of specific gravity 1.18 to 1.22 is used. This is prepared by adding 416.2 gm $ZnSO_4 \cdot 7H_2O$ to a 1000 ml volumetric flask. Distilled water is added, 100 ml at a time, until chemical is dissolved, then q. s. to 1000 ml and allowed to cool. More water is added if necessary after cooling. To test the specific gravity 100 ml of the above solution is weighed and should range between 1.18 and 1.22 gm/ml.

METHODS

1. Fecal samples are collected in plastic bags. Zinc sulfate solution is added to the feces in a 2 or 3:1 ratio and they are emulsified, strained, and poured into a plastic disposable test tube.
2. These are centrifuged 3 to 5 minutes at 2000 rpm.
3. The tube is removed and sufficient zinc sulfate added to bring the convex-meniscus over the top of the tube.
4. A 22 x 22 mm cover slip is placed on the top of each tube and allowed

to stand 10 to 15 minutes.

5. After standing, the cover slip and clinging liquid are placed on a slide for microscopic examination.
6. Slides are examined under both low and high power for ova identification.
7. Parasites are rated at 1+, 2+, 3+ and 4+ (1+, 0-1 per low power field, 2+, 1-3 per low power field, 3+, 4-6 per low power field, 4+, 6 to too many to count).

Rectal Swabs on Unweaned Puppies for Ova and Parasites

Procedures:

1. Each puppy in the litter is rectally swabbed with a cotton-tipped applicator soaked in 0.9N NaCl.
2. All swabs are placed in 1-2 ml $ZnSO_4$ solution (Sp. gr. 1.20) for 15 to 30 minutes.
3. Utilizing a 10 x 75 ml test tube a standard flotation is performed.

Baermann Technique for Strongyloides Larvae

Procedure:

1. A glass or plastic funnel with a diameter of 4 inches or greater is set up in a funnel rack with a short piece of rubber tubing attached and a hemostat closing the tubing.
2. A paper and gauze straining funnel, 6 inches in diameter, is placed inside.
3. The funnel is filled with distilled water to a level covering 2/3 of the gauze and a specimen of fresh stool is placed on the gauze.
4. After standing at room temperature for 5-7 hours a few drops of liquid are removed from the tubing and examined microscopically for larvae.

Table 7 presents the degree of infection as determined by the number of fecal samples that had ova or larvae present after processing through the proper procedure. This is based on 4591 fecal examinations. The percentage of positive samples is higher than desired but can be explained on the basis that 70 "outside" dogs were brought in for establishment of the breeding colony. The high percentage of Toxocara canis resulted from the large number of positive flotations found on weanling puppies, this parasite being transmitted by placental transfer. The larval migratory stage of this helminth and its ability to be reactivated during periods of stress, such as in pregnancy, is evidence for this.[2] Monthly fecal examinations and selective treatment have reduced this infestation in the colony during the past year. Table 8 presents the percentage of dogs that were positive for one or more internal parasites during the month of May 1965. It is divided into the FD dogs which were obtained from outside sources and LF dogs which have been raised here at the Laboratory. The data are based upon fecal examinations on 132 FD and 328 LF dogs. The majority of LF dogs upon which fecal examinations were performed were puppies from newborn to one year of age. Because of the high percentage of Toxocara canis infection in weanling puppies, fecal flotations are currently being performed during the first six weeks post partum. Fecal samples are being obtained by rectal swabs, a more reliable technique than attempting to collect fecal samples from within the cage. It is anticipated that this procedure will reduce the incidence of this parasite by virtue of early detection. This is of prime importance in order to reduce the stress to the developing pup.

The high incidence of Uncinaria stenocephala and Ancylostoma caninum is accounted for by the large number of positive flotations from dogs purchased from outside sources.

In December 1964, the rabidaform larvae of Strongyloides stercoralis were found in the fecal flotations of several litters. Positive diagnosis was made by extracting the suspicious fecals using the Baermann technique and culturing the extract in peat moss until all living forms, except the free

TABLE 7

Parasites	Positive Fecal	% of Sample Positive
Toxocara canis	248	5.4
Toxascaris leonine	101	2.2
Isospora sp.	82	1.8
Uncinaria stenocephala	26	0.6
Ancylostoma caninum	49	1.1
Trichuris vulpis	20	0.4
Strongyloides stercoralis	61	1.3
Totals	587	12.8

TABLE 8

Parasites	Positive Fecals on FD Dogs	% Dogs Infected	Positive Fecals on LF Dogs	% Dogs Infected	Total Positive Fecals	% of Total Colony Infected
Toxocara canis	8	6.1	53	16.	61	13.2
Toxascaris leonina	4	3.0	1	0.4	5	1.1
Isospora sp.	3	2.3	2	0.6	5	1.1
Uncinaria stenocephala	4	3.0	2	0.6	6	1.3
Ancylostoma caninum	1	0.7	2	0.6	3	0.07
Trichuris vulpis	1	0.7	0	0.0	1	0.02
Strongyloides stercoralis	0	0.0	0	0.0	0	0.0

living male, were identified. As recorded in Table 7, there were 61 positive determinations for this larva. These were from 10 dogs purchased from outside sources and 51 puppies from 21 litters. During the month of February 1965, all animals within the colony had a fecal examination for Strongyloides stercoralis performed by one zinc sulfate flotation and three consecutive Baermann analyses. Larval forms of this organism were detected in 39 dogs on the original zinc sulfate flotation, 15 dogs on the first Baermann analysis, 7 dogs on the second Baermann analysis and no dogs on the third Baermann analysis. All of the infected dogs, their dams and cage mates, were isolated in one kennel. These animals were treated daily for 30 days with 10 ml of Dithiazanine Iodide (Dizan, Corvel, Inc., Omaha, Nebraska) per pound body weight. They were clinically examined each day during this period and no determinable side effects were observed. Fecal flotations and Baermann extractions following this treatment have indicated no reinfestation of the animals as of this date. Of the 21 litters in which there was infestation of the larva, none of the dams of these litters were positive for the larval form of this organism.

ESTRUS EXAMINATIONS

Procedure:

1. Materials.

   The bottom 30 mm of a 13 x 100 mm test tube are etched until opaque. The tube is then placed into an autoclaving envelope and sterilized before use.

2. Technique.

   a. The test tube is inserted about 40 mm into the vagina of the bitch, rotated and withdrawn.
   b. Immediately the vaginal material adhering to the tube is rolled onto one or two slides.
   c. The slides are allowed to air dry and then stained with Wright's stain before examining.

3. Examination.

The slide is examined to determine the following stages of the reproductive cycle:

a. Proestrus

This stage extends from the first appearance of blood from the vulva to the time of first acceptance of the male. The duration of this period is from seven to nine days. On examination the slide shows few or no leukocytes, the epithelial cells are cornified and there are numerous erythrocytes.

b. Estrus

This is the period of acceptance of the male and may vary from five to twelve days, averaging about nine. The slides show an absence of leukocytes and fewer erythrocytes than in proestrus. Cornified epithelial cells stain dark with indistinct nuclei and are vacuolated and disintegrating. They appear in the form of boat cells. Bacteria may be present. The bitch is ready for breeding.

c. Abnormal Condition

During the estrus periods several slides should be examined for pyometria or vaginal infection. Such infections would be characterized by a large number of granulocytic leukocytes, superimposed upon the normal picture of estrus.

PROGRESS

With information obtained from vaginal smears, it has been possible to maintain the colony breeding efficiency above 90%, with minimal usage of the studs. These smears have helped in the early detection and treatment of vaginal and uterine infections and have prevented loss of a number of breeders due to metritis.

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INVESTIGATION OF THE CAUSES OF PRENATAL AND EARLY POSTNATAL DEATH IN BEAGLES

by

H. C. Redman

INTRODUCTION

For any long term investigation it is necessary to utilize an animal that is in good health, and it is important to have this animal biologically assessed periodically from birth to death. The fission-product studies require the production of the 300 beagle dogs per year and therefore it is necessary to maintain a breeding colony of approximately 60 adult females. From a scientific and economic standpoint it is important to investigate continually the causes of death in the newborn pups. Previous studies have been conducted on a limited basis,[1-4] and have indicated a very high mortality during the first 48 hours. These investigations have documented causes of death arising from stillbirth, congenital malformations, pneumonia and early accidental deaths. Mortality within these colonies varied from 16 to 42 per cent. Hopefully, with the determination of causes of death in the newborn, preventive steps can be taken to reduce the mortality rate to a minimum.

METHODS

An investigation into the causes of death was initiated in the breeding colony in September 1963, but the colony then was small and had limited production until May 1964. When a puppy died a gross post mortem examination was performed as soon as possible by a veterinarian. Tissues were collected and sent to the Department of Pathology for routine histopathological examination. These consisted of heart, lungs, liver, kidney, intestine and, with discretion, other tissues or organs. Bacteriological

cultures are routinely obtained in order to determine the bacterial flora present in the hope that these might be related to causes of death. Sections of heart, lung, liver, kidney and bladder are now also being routinely screened for viruses. Additional future studies are anticipated for immunological investigations of the bitch and puppy for more prevalent canine diseases, e.g., canine distemper, infectious canine hepatitis and leptospirosis.

RESULTS

Presented herewith are results of investigating the first 91 litters. Table 1 gives the beagle production rate and in Table 2 the causes of death are noted using absolute and percentage figures. These were determined on the basis of medical history, gross post mortem examination and histopathological examination. The age at death is tabulated in Table 3.

Table 1

BREEDING COLONY PRODUCTION

Number of Litters	Number Whelped	Number of Dogs per Litter
91	473	5.2

Number Died	Number Weaned	Number Weaned per Litter
73	400	4.4

Percentage of mortality to weaning 15.4%

Table 2

PUPPY MORTALITY TO SIX WEEKS OF AGE

(91 Litters)

Cause of Death	Number of Deaths	Percentage of Mortality
Parturition		
Dead Birth	4	5.5
Incomplete Pulmonary Expansion and Suffocation	24	32.9
Maternal Trauma	4	5.5
Congenital Anomalies		
Cleft Palate	2	2.7
Didactylism (abnormal pharynx)	1	1.4
Patent Ductus Arteriosus	2	2.7
Infection		
Pneumonia (all causes)	11	15.1
Omphalophebitis	7	9.6
Accident	2	2.7
Other (toxicity)	6	8.2
Undetermined	10	13.7
	73	100.0

Table 3

PUPPY MORTALITY TO SIX WEEKS OF AGE

(91 Litters)

AGE AT DEATH	NUMBER OF DEATHS	PERCENTAGE OF MORTALITY
Less than 24 hours	32	43.8
1 day	5	6.8
2 days	3	4.1
3 days	7	9.6
4 days	2	2.7
5 days	1	1.4
6 days	1	1.4
7 days	0	0
8 days	0	0
9 days	0	0
10 days	2	2.7
11 days	0	0
12 days	3	4.1
13 days	2	2.7
14 days	0	0
15 days	2	2.7
16 days	0	0
17 days	2	2.7
18 days	2	2.7
19 days	1	1.4
20 days	0	0
21 days	0	0
22 days	0	0
23 days	0	0
24 days	0	0
25 days	1	1.4

Table 3 (cont.)

AGE AT DEATH	NUMBER OF DEATHS	PERCENTAGE OF MORTALITY
26 days	0	0
27 days	0	0
28 days	0	0
29 days	0	0
30 days	0	0
31 days	0	0
32 days	0	0
33 days	0	0
34 days	0	0
35 days	0	0
36 days	0	0
37 days	0	0
38 days	0	0
39 days	0	0
40 days	1	1.4
42 days	0	0
Over 6 weeks	6	8.4
	73	100.0

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ISOLATION OF A PASTEURELLA SPECIES FROM BEAGLE PUPPIES AND BROOD BITCHES

by

J. H. Sherrod and E. Kinter

INTRODUCTION

A species of Pasteurella was isolated from specimens taken at gross autopsy of puppies surveyed in an investigation of the causes of neonatal deaths in beagles. These animals revealed a significantly higher than expected incidence of patent ductus arteriosis and other greater artery anomalies. Since the publications of Jawetz describing Pasteurella pneumotropica,[1] there have been other reports of finding this organism in various animals.[2-5] However, very little has been published on its detection in dogs[6] There have been no publications found that implicated Pasteurella spp. as an etiological agent of heart and blood vessel anomalies. Studies have been published on neonatal death and greater vessel anomalies with various causes attributed from congenital defects to viruses.[7-18] Nothing in these papers suggest the etiology to be Pasteurella pneumotropica.

METHODS

Puppies: Particular emphasis was given to sampling the heart muscle and valves, the patent ductus arteriosis (if present) and lining scrapings of the greater arteries for bacteriological culture. Routine cultures were also taken from the lung, liver, kidney and gastrointestinal tract.

Maternal bitches: Vaginal swab cultures were taken from animals showing metritis, prolapsed vagina and abnormal vaginal discharge. Placental cultures were obtained.

Males: Preputial swab cultures were taken from several male dogs of the breeding colony.

Culture procedures: Cultures were inoculated to blood agar plates and Trypticase Soy Broth (Baltimore Biological Laboratory, Baltimore, Maryland) tubes. Organisms were picked to pure cultures and routine identifications made. Confirmation of Pasteurella spp. was made by Miss Elizabeth King of Communicable Disease Center (CDC), Atlanta, Georgia.

RESULTS

An organism culturally like Pasteurella pneumotropica was isolated and confirmed in 26.8% of 41 puppies autopsied. Patent ductus arteriosis of varying degrees of severity (admitting free passage of probes from 3 cm. to 10 cm. in diameter) was found in 15 of this group of animals. Isolations of this organism have been made from the placental stump of the umbilical vein and samples of the cut surface of the vascular portion of the placenta. Histological sections of tissue from puppies from which this organism was isolated showed areas of clumped bacteria, Polymorphonuclear (PMN) infiltration and connective tissue proliferation.

This same Pasteurella organism has been isolated in all 95 vaginal cultures of bitches in proestrus, as well as in all of 39 postpartum vaginal cultures from bitches in the breeding colony. In addition, this organism has been isolated in six proestrus cultures from bitches outside the breeding colony.

Isolation of this organism has been made in 27 cultures from bitches showing hemorrhagic vaginal discharge. Among the clinical conditions exhibited by these animals were metritis, vaginal prolapse, perforated laceration of the vaginal wall and hemorrhagic discharge during gestation. So far, this organism has not been isolated from any of these bitches during anestrus.

A greater predominance of this organism in bitches was observed to be in direct relationship to the amount of macroscopic blood in the vaginal discharge.

Coital contamination cannot be ruled out in the preputial isolations made.

CONCLUSIONS

It would seem possible that this Pasteurella spp. is involved in the etiology of a high incidence of patent ductus arteriosis and other greater vessel anomalies. Further investigation is now being conducted to develop a serological identification and screening test; studies are also being made to determine an effective clinical treatment.

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INVESTIGATION OF PUPPY DIARRHEA IN BEAGLES

by

J. H. Sherrod and E. Kinter

This investigation was initiated to study a clinical hemorrhagic diarrhea observed in beagle puppies shortly after weaning. This diarrhea is accompanied by a loss of weight, decreased appetite and general unthriftiness. In general, this syndrome closely resembles calf scours of Escherichia coli origin which suggested the possibility of this being a contributing factor.

METHODS

In an attempt to determine if Escherichia coli had pathogenic potentialities in dogs, a series of fecal cultures were taken from puppies showing diarrhea with macroscopic blood. Fecal samples were inoculated to blood agar plates, Eosin Methylene Blue, EMB, (Difco Laboratories, Detroit, Michigan) and Trypticase Soy Broth (Baltimore Biological Laboratory, Baltimore, Maryland). Numerous picks were made for pure culture. Cultures were checked with Escherichia coli OB polyvalent antisera groups A and B used for identification of pathogenic types predominant in humans. Those showing agglutination were verified by chemical reactions. Further attempts were made to identify the O group by use of live and boiled antigens.

PROGRESS REPORT

The majority of the 156 cultures examined have shown agglutination in the polyvalent B group. There have been several cultures which appear to agglutinate both groups equally well. With the antisera used, it would indicate a common B group. As yet we have been unable to determine the specific O groups involved in these episodes.

A literature search has been made and has revealed no evidence of serotypes most commonly found in dogs (see Bibliography).

Investigation is continuing to identify the specific antigenic groups of Escherichia coli in these animals. Consideration is also being given to isolation of other possible causes of puppy diarrhea.

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ARTIFICIAL INSEMINATION IN THE BEAGLE

by

J. H. Sherrod, N. Pollock and M. Wilson

PURPOSE AND METHODS

The purpose of this study is to extend the use of the limited number of available studs in the colony and to bring into use other studs adequately tested but not formally introduced into the colony. Two studs tested for fertility and proven sires by natural breeding were selected for use; one was a colony stud and the other was from outside the colony. Six bitches were bred during the year in two separate attempts at artificial insemination. The semen ejaculate was collected in a sterile glass tube by means of manual manipulation of the dog's penis after he was teased with a bitch in estrus. Immediately following collection a small sample of the semen to be examined was removed from the tube. A plastic cap was placed on the tube and the remainder of the sample was placed in an 80° F. waterbath until the bitch to be inseminated was readied and placed on the table. As soon as the bitch was placed on her back on an examining table, the semen sample was removed from the test tube and 2 ml of undiluted semen was placed in a sterile syringe. To the syringe was attached an 18-inch long bovine insemination catheter constructed of plastic. With the use of speculum, the cervix was exposed and the catheter inserted into the cervical vestibule. The entire content of the syringe was deposited in the uterus with as little pressure as possible to avoid back leakage into the vagina. After withdrawing the catheter and speculum the bitch was held on her back with hindquarters elevated for five minutes.

RESULTS

None of the inseminations resulted in pregnancy in the bitches. One explanation for the first failure was the use of a stainless steel insemination catheter instead of the plastic one presently employed. Subsequent

tests with these catheters proved that upon contact with metal motility of the sperm quickly ceases. In the second attempt, unproven bitches were used and it is possible that the bitches were temporarily or permanently sterile.

Hopefully, continuation of this work during the coming year using selected proven bitches, semen expanders and buffers, will prove successful.

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PRELIMINARY INVESTIGATION OF THE USE OF ELECTRICAL ANESTHESIA ON BEAGLES

by

J. H. Sherrod

This study was initiated to find a suitable method of restraining animals during inhalation exposure to various aerosols. It was also desirable to find a method (with minimal adverse effects) for use in restraining animals for routine clinical and radiographic examination, particularly in debilitated or aged animals that would not tolerate sedation with clinical anesthesia. Reports of the use of electrical anesthesia in dogs and other animals suggested that this might satisfactorily serve our purpose.

Trials were conducted using two types of apparatus. The first, fabricated in our laboratory, delivered pulses superimposed on a direct current (dc) of like polarity. The second, manufactured by Electronic Medical Instrument Co., Loveland, Colorado, delivered sinusoidal alternating current (ac).

Various electrode placements were tried on the heads of animals: 1) bitemporal; 2) frontal-occipital; 3) palatine-occipital and 4) orbital-occipital.

Preliminary results have been encouraging; in over 200 successful attempts the ac unit has given more consistent results than the pulse-dc combination. However, the electrode placement is apparently one of the most important success factors. Midline frontal-occipital location of the electrodes has given the best results. Clinical - surgical anesthesia has been achieved using final current settings averaging 16 milliamperes with an average frequency of 3200 cycles-per-second ---- depending upon the individual animal. The most effective method of attaining anesthesia with the ac unit has been to alternately increment the current from zero and the frequency from 2000 cycles-per-second. When care is exercised in these rates of increase, muscle spasms, respiratory arrest, defecation and

urination are not a problem. However, it must be pointed out that the conventional indexes of surgical depth (i.e., corneal and foot-pad reflexes) may not be accurate in assessing this type of anesthesia.

Further investigation is planned using this method of anesthesia. Consideration will be given to physiologically monitoring the animals before, during and after anesthesia. The possibility of two simultaneous ac waveforms will also be studied.

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EFFECTS OF METAPHASE ARRESTING DOSES OF COLCHICINE ON TISSUE ENZYMES

by

T. L. Chiffelle, R. K. Jones and J. K. Scott

The alkaloid colchicine has been widely used for many years in studies of cell turnover rates and of chromosomal analysis. In proper dosage, the drug will arrest in metaphase all cells that enter division during the period between administration of colchicine and the time of its elimination (approximately 6 to 8 hours).[2] In addition to mitotic arrest the drug produces a temporary leukopenia in moderate doses.[2, 6, 7] In large doses, after a latent period of 3 to 6 hours, the drug induces widespread toxic effects particularly to the gastrointestinal tract and the central nervous system. A hemorrhagic gastroenteritis may be produced, with fluid and electrolyte loss similar to the effects of arsenic or radiation. Hematuria and oliguria may result from injury to the renal excretory system. Effects on the central nervous system are poorly understood but include exhaustion, muscular weakness, ascending paralysis, convulsions and respiratory arrest. Death seldom occurs before 12 hours and some animals may survive two weeks with toxic doses.[4, 7] A wide variation of dose for toxic manifestations exists between species and between individuals within the species.[3, 5] Some difference in toxicity has been described for different methods of administration. Apparently the amount of drug necessary to arrest mitotic activity adequately at metaphase is near or at the toxic level.[1]

The accumulation of arrested mitotic figures approached linearity from 30 minutes to 6 hours after injection and thereafter it sharply decreased.[2] Chemical assays for the drug in marrow have shown a parallel increase in concentration to 4 hours, after which the concentration declines and disappears in 8 hours. Thus, the effect on marrow elements is very prompt.

Although the toxic effects of colchicine have been known for some time, it seemed logical to study these in the course of treating our beagle dogs hours prior to sacrifice to obtain marrow cellular elements arrested in metaphase, for appraisal of chromosomal aberrations after exposure to Sr-90. The amount of drug administered to dogs was extrapolated from the dose previously administered to rats (3mg. per kilogram) for the same purpose. In a pilot study using beagle dogs, averaging 10 kilograms and 2 to 3 years of age, it became apparent that the original dose used was nearly 70 times the toxic level. After surveying the results of this pilot study, it became apparent that the doses of colchicine required to produce sufficient numbers of arrested mitotic figures for chromosome studies may cause an effect on blood constituents and on tissue enzymes to be assessed histochemically at the time of sacrifice (2 to 3 hours later). An experiment was designed using rats (male and female) to assess hematologic, histochemical and histological effects of graded doses of colchicine at various times after injection. According to Ferguson the LD_{50} for rats is 1.7 mg. per kilogram.[4]

METHODS

Eighty Holtzman rats, 150-200 grams weight, were divided into 5 groups of 16 animals each (8 males and 8 females). Each rat was given a subcutaneous injection of colchicine in physiologic saline in doses of 0.5, 1.0, 2, 0, 3.0, and 4.0 mg. per kilogram. Twenty animals (4 to each group, male and female) were for hematologic studies. The remaining 60 animals (12 in each group, equal males and females) were used for chromosomal studies, tissue enzyme studies and general histopathologic analysis. Twenty additional rats (4 to a group) were controls. In each dose group, equal numbers of male and female animals (2 each) were anesthetized and sacrificed at 2, 4 and 6 hours after injection.

Chromosomal preparations were made from femoral marrow, by methods described previously,[8] for studies of mitotic incidence at various intervals after injection and also for the quality of chromosomal spread for appraisal of morphologic aberrations. Thin slices of kidney,

liver, intestine and thyroid gland were quick frozen by quenching in isopentane at liquid nitrogen temperature for histochemical analysis of alkaline and acid phosphatase, succinic dehydrogenase and DPNH diaphorase according to methods described previously.[8] Tissue sections of a wide variety of organs from each animal in each group were fixed in 10% buffered formalin and processed for general histologic survey.

TENTATIVE RESULTS

The higher level doses of 3 and 4 mg. per kilogram produced many chromosomes in both the 2 and 4 hour sacrifice groups. Analytically, however, there was some slight variation between animals within this period. In spite of this large number of chromosomes, there was much overlapping of the individual chromosomal groups resulting in a less-than-desirable preparation for detailed morphologic analysis. In the 2 mg. per kilogram range, the chromosomal preparations were of excellent quality, without overlapping and with good definition of the individual chromosomal structures. There appeared little difference between the preparations made in the 2 and 4 hours sacrificed animals.

At a dose of 1 mg. per kilogram there was marked reduction in the number of cells at metaphase compared with the results obtained at higher doses in those sacrificed at 4 hours. Specimens taken at the 2 hour interval were unsatisfactory. Marrow preparations taken from animals having received the lowest dose (0.5 mg. per kilogram) showed no effect. At all dose levels above 0.5 mg. per kilogram, preparations made from the 6 hour sacrifice specimens showed that cells arrested in metaphase were markedly diminished in number and that quality of the preparations was poor.

For a young rat, subcutaneous injection of colchicine (2 mg. per kilogram) resulted in an optimal number of chromosomes at either 2 or 4 hours post-injection. This dose level produced the most satisfactory chromosome preparations, particularly when compared to preparations made at the two higher dose levels. It is possible that the quality of chromosomal preparations in the higher dose ranges might have been more

satisfactory at an earlier sacrifice (1 hour). Cytotoxic effects of colchicine have been well described and large doses may exert some effect on the ability of a cell to divide. Also, large doses of the drug may induce abnormal mitotic figures.[6,7] Insufficient material has been examined at this time for histochemical enzyme changes or general histopathologic effects to be discussed in this report. Additional results are forthcoming.

AUTO-ANTIBODY PRODUCTION IN THE DOG

by

G. H. Meade, J. H. Sherrod and W. E. Clapper

ABSTRACT

Injection of homologous kidney with Freund's adjuvant, with E. coli endotoxin and the toxin and adjuvant without kidney produced low titres of complement fixing antibodies. No antibodies were shown by the tanned cell hemagglutination or gel diffusion methods.

I. Antibody Production to Homologous Kidney Tissue in Beagles

The production of antibodies to the host's own tissue is indicative of damage to or change in this tissue. Radiation causes damage to exposed tissues, so it seems possible that antibodies might be produced as a result. Such antibodies might even be detected in the absence of other signs of radiation damage, and thus serve as an early indicator. They might also be the forerunner of further damage to specific organs that would be a part of the radiation syndrome.

Antibodies have been produced in other animals by injection of tissues from one of the same species along with toxins or adjuvants.[1,2] The dog has been reported to make antibodies to soluble protein antigens with difficulty.[3] As a beginning in the study of this phenomenon in beagles exposed to radioactive aerosols, it seemed necessary to first determine whether they could produce antibodies under controlled conditions.

Dog kidney was processed according to a method adapted from one used by Stebley and Lepper.[4] This produced a suspension containing 50 mg/ml of glomerular basement membrane. This was used both for immunization and for skin testing the experimental animals. The following experimental design was used:

	No. of Dogs	Amount of Tissue	Adjuvant
Group 1	2	None	None
Group 2	2	None	E. Coli toxin
Group 3	2	None	E. Coli toxin + Freund's
Group 4	2	37 mg.	Freund's
Group 5	2	37 mg.	E. Coli toxin

Injections were made intradermally in the flank, intramuscularly in the semi-tendinsus-membranosus muscle group and subcutaneously in the cervical area. Booster injections of the same amounts of material were given 3 weeks later. Skin tests consisting of 10 mg of antigen injected intradermally were done at 3, 4 and 5 weeks and at 2 months following the initial immunizing dose. These tests were read at 2, 24, 48 and 72 hours. Blood was drawn before the first injections and at 3, 4, 5, 9 and 15 weeks thereafter. The serum was examined for antibodies against dog kidney tissue using the gel diffusion, tanned cell hemagglutination and complement fixation techniques. Serum made by immunizing rabbits with dog kidney was found to be positive by all of these methods but was organ specific only for complement fixing antibodies. Results of the antibody determination by this method are seen in Table 1. Gel diffusion and tanned cell hemagglutination titres were negative for all specimens taken. Skin tests were positive at only one period (5 weeks). At this time, one dog in group 3 showed a slight erythema at site of test injection at 2 hours. At 24 and 48 hours this raised reddened area had increased to 3 cm in diameter.

The results of this experiment gave suggestive evidence that antibodies to homologous kidney can be produced in the beagle. It appears that coli toxin alone can induce the production of kidney antibodies. To determine whether a more intensive immunization program could produce higher titres, two more dogs were injected at weekly intervals for 5 weeks with emulsions of equal amounts of dog kidney tissue and Freund's adjuvant. The complete adjuvant was gradually diminished until the final inoculation was made with equal amounts of the tissue and Freund's incomplete adjuvant. No antibodies to kidney were found in these animals 10

Table 1

Complement-fixing Antibody Titer in Dogs Injected with Homologous Kidney Tissue

Dog #	Time of Sampling: Pre Injection	3 weeks	4 weeks	5 weeks	9 weeks	12 weeks
Control B8	0	0	0	0	0	0
Control B4	0	0	0	0	0	0
Coli toxin B16	0	0	0	0	0	0
Coli toxin 137F	0	1-32	1-64	1-32	1-16	1-16
Coli toxin + Freund's 146C	0	1-16	1-32	1-32	1-32	1-32
Coli toxin + Freund's B14	-*	1-16	1-16	1-32	1-16	1-16
Kidney + Freund's 60A	-*	1-32	0	1-16	1-16	1-16
Kidney + Freund's F40	0	1-16	1-32	1-64	1-32	1-64
Kidney + Coli toxin V52	0	1-32	1-64	1-64	1-32	1-32
Kidney + Coli toxin 34	0	1-32	1-64	1-64	1-32	1-32

*No serum available. The highest concentration of serum used was 1-16 since 1-8 was often anti-complementary. Values represent the median of 3 or 4 determinations.

days after the final injection. Further attempts should be made to determine the most sensitive immunological procedure to use to test for these antibodies, the method of preparing the most active antigen and the period at which antibody is at its height. Further attempts to determine fixed antibodies should also be made.

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AUTO-ANTIBODY PRODUCTION IN THE DOG

by

G. H. Meade, J. H. Sherrod and W. E. Clapper

ABSTRACT

Serum from beagles in which a single kidney had been damaged by X-rays was tested for complement fixing and tanned cell hemmagglutinating antibodies. E. coli toxin was also administered to 2 of these dogs. A slight rise in complement fixing antibodies was observed in the irradiated animals. Use of the animals own kidneys for antigen did not show either type of antibodies in the serum of the dogs from which they had been taken.

II. Production of Antibodies by Exposure of the Kidney to X-Irradiation.

Eight beagles were used in this experiment to determine whether a damaging dose of X-irradiation to the kidney would produce kidney antibodies. Two dogs served as controls, two were injected with E. coli 055: B5 toxin (Difco Laboratories, Detroit, Michigan) as described in article I, two were injected with E. coli toxin and the right kidney of each exposed to X-ray; and two had the right kidney exposed to X-ray only. The distance from the specimen to the center of the tube was 48 cm. Fifty-eight roentgens (air dose) per minute were delivered for 20 minutes at this distance for a total of 1024 r.* The coli toxin was used because in irradiated animals the toxin from coliform organism in their own intestine may leak into the blood stream. This could possibly enhance kidney damage. Blood was drawn prior to treatment and at 3, 4, 5, 6, 8, 12, 16 and 20

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* X-irradiation and dosage measurements were kindly performed by Dr. J. de Boer, Radiobiology Group, Air Force Weapons Laboratory, Kirtland Air Force Base.

weeks. The separated serums were stored at -60° C until tested. Tanned cell hemagglutination studies showed no antibodies to kidney antigen. The results of the complement fixation tests are shown in Table 2.

A slight rise in titre was observed in the dogs exposed to X-rays; the reliability of the results was questionable because of the anti-complementary activity of many of the specimens. This activity could not be eliminated in some cases by either incubation at 60° C or addition of complement and subsequent destruction of the complement by heating.

Nine months following injection and exposure, four dogs were sacrificed and the kidneys removed. Tissue from the middle of the unexposed kidney from each animal was sent to the Pathology Department for examination. This was done to determine whether immunological changes might be found in these organs suggestive of the formation of antibodies against kidney. The pathological findings were as follows:

> The unexposed kidney from one of the dogs subjected to X-irradiation and an injection of E. coli toxin showed histo-pathological changes. The renal tissue was reported to exhibit "Marked capsular thickening, nodular mesangial thickening and fibrosis, and tubular atrophy with formation of multiple small cortical scars".

Antigens were prepared from the exposed and unexposed kidneys of the four dogs, as described by Rose et al.[1] Two of these beagles had been injected with E. coli toxin only. Tests using the dogs own serum against antigens prepared from each of its own two kidneys were made by complement fixation and tanned cell hemmagglutination techniques. It was thought that antibodies might be produced which were so highly specific that they would react only with the autologous kidney. However, no such antibodies were found. It is perhaps possible that the antigens made from these experimental animals were already saturated by antibodies produced in that animal.

Possibly circulating antibodies are not produced under the conditions of this experiment. It is possible that they are immediately removed from circulation and are fixed in the kidney. Some evidence for this type

Complement-fixing Antibody Titres to Kidney in Beagles Exposed to X-Irradiation

Table 2 Time of Sampling

Dog	Test #	Pre treat-ment	wk. 3	wk. 4	wk. 5	wk. 6	wk. 8	wk. 12	wk. 16	wk. 20
75 Control	1 2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
77 Control	1 2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
76 Coli toxin	1 2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
78 Coli toxin	1 2	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
73 X-ray	1 2	0 0	0 1-16	0 1-16	0 0	1-16 1-16	1-16 1-16	0 1-16	1-16 1-16	1-16 0
74 X-ray	1 2	0 0	0 1-16	1-16 0	1-16 0	1-16 0	1-32 0	1-32 0	1-16 1-16	1-16 1-16
71 Coli toxin X-ray	1 2	0 0	0 1-16	0 0	1-16 1-16	1-16 1-16	1-16 0	1-16 0	1-16 1-16	1-16 0
72 Coli toxin X-ray	1 2	1-16 0	1-16 1-16	1-16 0	1-16 0	0 0	0 1-16	0 1-16	0 1-16	1-16 1-16

of reaction has been offered by others[2] and the anti-complementary activity of dog blood has been noted.[3] It has been suggested that a titration of this activity may be related to immune response.[4]

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AUTO-ANTIBODY PRODUCTION IN THE DOG

by

G. H. Meade, J. H. Sherrod and W. E. Clapper

ABSTRACT

Initial body burdens between 31 and 46 microcuries per kilogram of Sr-90 were introduced into dogs by inhalation. These dogs produced no detectable levels of complement fixing or tanned cell hemagglutinating antibodies to dog kidney or thyroid. Eight beagles were exposed and 8 were used as controls.

III. Antibodies to Kidney and Thyroid in Beagles Exposed to Sr-90

An over-all study of the long term effects of inhalation of radioactive particles is being conducted by several Departments of The Lovelace Foundation. The purpose of the work reported in Sections I and II of this series was to carry out exploratory work related to the formation of auto-antibodies in the beagle. Although no highly definitive results derived from the preliminary experiments, it was felt that animals exposed to irradiation for a prolonged period such as that produced by inhaling radioactive particles should be examined for circulating "auto-antibodies". The methods with which we had previous experience were used; others have thought these (complement fixation and tanned cell hemagglutination) to be most sensitive and they also would serve as a measurement of the two different kinds of antibodies.[1] Thyroid antibody studies were included because these have been found in animals exposed to ionizing radiation.[2]

Sixteen dogs were used for this and other microbiological experiments. Eight served as controls. Eight inhaled a Sr-90 aerosol and had total body counts indicating 31-46 microcuries per kilogram. Blood for serum samples was taken weekly for the first month, biweekly for the next 6 weeks and monthly thereafter for 6 months. Antiserum to dog thyroid, kidney, heart, lung and liver was produced in rabbits for control

serums.

All serum specimens were tested for complement fixing antibodies against dog kidney. The results were essentially negative. Tanned cell hemagglutinating antibodies to kidney were not found in any of the specimens. Serums from two exposed dogs showed no hemagglutinating antibodies to heart, liver or lung antigens. All specimens were tested for complement fixing antibodies against thyroid and found to be negative. To date, all serums from 2 exposed and 2 control dogs have shown no agglutinating antibodies for thyroid. The others will be tested. Dogs exposed to a higher level of Sr - 90 will be tested in a similar manner since this dose achieved no observable changes.

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THE WHOLE BODY RETENTION OF INHALED Cs-137 IN BEAGLE DOGS

by

Bruce B. Boecker

ABSTRACT

The whole body retention of a tracer level of Cs-137 deposited during a single, short inhalation exposure has been followed in two groups of dogs. During the first experiment (25 dogs), the diet was changed by adding raw ground beef plus a vitamin and mineral supplement to the commercial food being used. Retention results from 8 dogs that were followed for 4 to 8 months after this diet change are examined. The mean value for the biological half-life, T_b, just prior to the diet change was 42 days. After the diet change, the values of T_b for 6 of the 8 dogs decreased such that the mean value became 36 days. A second experiment with 5 dogs that will be fed the same diet for the duration of the experiment is now in progress. At 100 days post-exposure, the mean value of the long term T_b is 30 days but considerable variation among these dogs has been observed. These variations cannot be attributed to age differences.

INTRODUCTION

The first inhalation experiment conducted on dogs at this Project involved single exposures to a Cs-137 Cl aerosol. Its purpose was twofold: 1) to determine the normal distribution and excretion patterns of inhaled Cs-137 in the dogs and 2) to test all exposure and metabolism procedures. A group of 25 dogs was used in this experiment, some of which were followed for one year post-exposure. When this study was completed a second group of 5 dogs was exposed in the same manner but housed under different conditions. This group is currently under observation and also will be followed for a year post-exposure. The whole body counting data

obtained from these 2 experiments will be discussed here. Companion tissue distribution and excretion data are being analyzed and will be presented later.

METHODS

The 12 male and 13 female dogs used in the first experiment were purebred beagles ranging in age at exposure from 17 months to an estimated 72 months. Some were raised locally and some were supplied by other AEC Contractor laboratories. They were placed in individual cages at least one month prior to exposure and were kept in these cages after exposure until they were sacrificed at 0, 1, 4, 8, 16, 32, 64, 128, 256 or 366 days post-exposure. At the start of this experiment, the dogs were fed Wayne Dog Food (Allied Mills, Inc., Chicago, Ill.) moistened with water. During the course of this experiment (on $\approx$ June 1, 1964), food mixing equipment became available and the diet of the entire adult dog colony was changed. From this date on, the dogs were fed a daily 300 gram ration containing the following percentages by weight: 60% Wayne Dog Food, 20% raw ground beef, 20% water and a small amount of Vionate® vitamin and mineral supplement (E. R. Squibb and Sons, New York, N. Y.).

The second group of dogs consisted of 2 male and 3 female beagles whose ages at exposure ranged from 19.0 to 20.2 months. They were placed in metabolism cages 14 days before exposure, kept there until 30 days post-exposure and then transferred to the kennels. Their diet throughout the experiment has been the standard mixture of Wayne Dog Food, ground beef, water and Vionate®.

Two or 3 dogs were exposed in any one day. Before exposure each dog was given 60 mg. of phenobarbital orally followed 105 minutes later by an intravenous injection of Vetame® veterinary tranquilizer (0.25 mg/lb) (E. R. Squibb and Sons, New York, N. Y.). The exposures were of 10 minute duration and involved an aerosol generated from a 1.0 per cent stable cesium chloride solution containing Cs-137. The mean air concentration ranged from 2.8 - 9.5 μc/L and the aerodynamic mass median diameter ranged from 1.6 - 2.0 μ for these exposures.

Each dog was whole body counted immediately after exposure and at selected intervals thereafter. The dogs were placed unsedated, in a plastic box and counted with a vertical whole body counter using a NaI detector or with a large volume, liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.) depending on the radioactivity levels involved. These counters are shown in Figures 1 and 2. Initial body burdens in these dogs ranged from 13 to 130 μc. During the early phase of the experiment, the counting was done on the vertical whole body counter with a separation of 109 cm. between the top of the crystal and the bottom of the counting box. With this configuration, Cs-137 distributed within a dog is detected with an efficiency of 0.024 per cent. Later in the experiment, when the body burdens had decreased somewhat, the counting was done at a crystal-to-box separation of 84 cm. (0.038 per cent efficiency). Finally, when the body burdens dropped below 2 μc, the remaining whole body counting was done with the liquid scintillation detector (23 per cent efficiency).

RESULTS

The whole body retention of Cs-137 was examined for each dog by fitting smooth curves by eye to the whole body counting data. Retention values for each day post-exposure for each living dog were taken from these curves and tabulated. An arithmetic mean retention was then calculated for each day post-exposure and these results are shown as individual points in Figure 3. A smooth curve was fitted by eye to these data and its equation was determined by the graphical subtraction method to be:

$$R_t = 8e^{-.693t} + 15e^{-.099t} + 77e^{-.016t}$$

where R_t is the whole body retention at time t expressed as a percentage of the initial body burden. The three exponents represent effective half-lives (T_e) of 1.0, 7.0 and 43 days. Since the physical half-life of Cs-137 is 30 years[1], the biological half-lives, T_b, are essentially equal to the values of T_e observed during these 1-year experiments.

At approximately 85 days post-exposure the experimental points break away from the smooth curve in a downward direction indicating an increased rate of excretion of the Cs-137. This result can be studied more

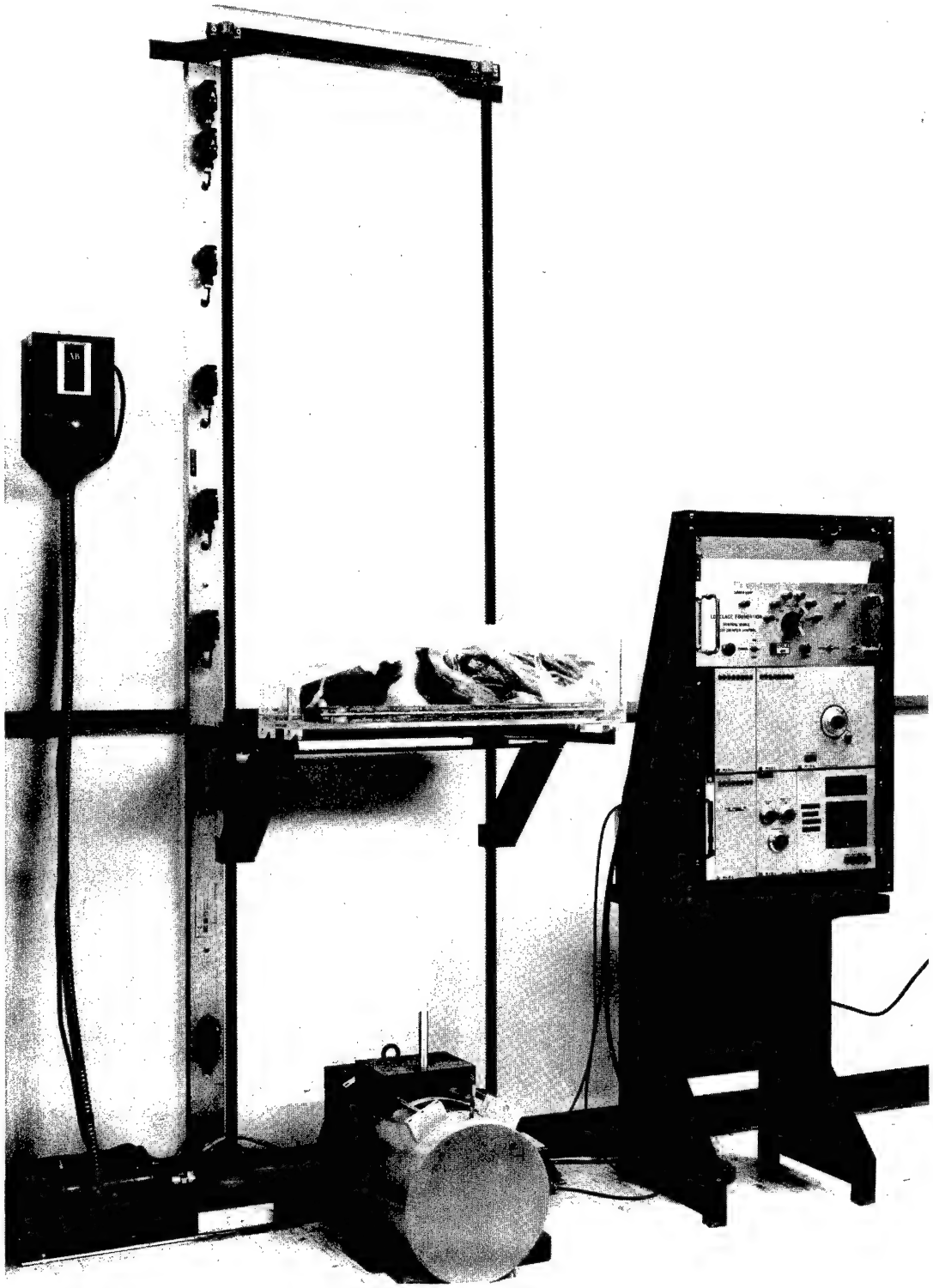


Figure 1: Dog being counted on veritcal whole body counter. Cylindrical lead shield contains 4" x 4" NaI crystal. Control panel for automatic raising or lowering of the dog is installed above the scaler and timer in the console to the right of the counter.

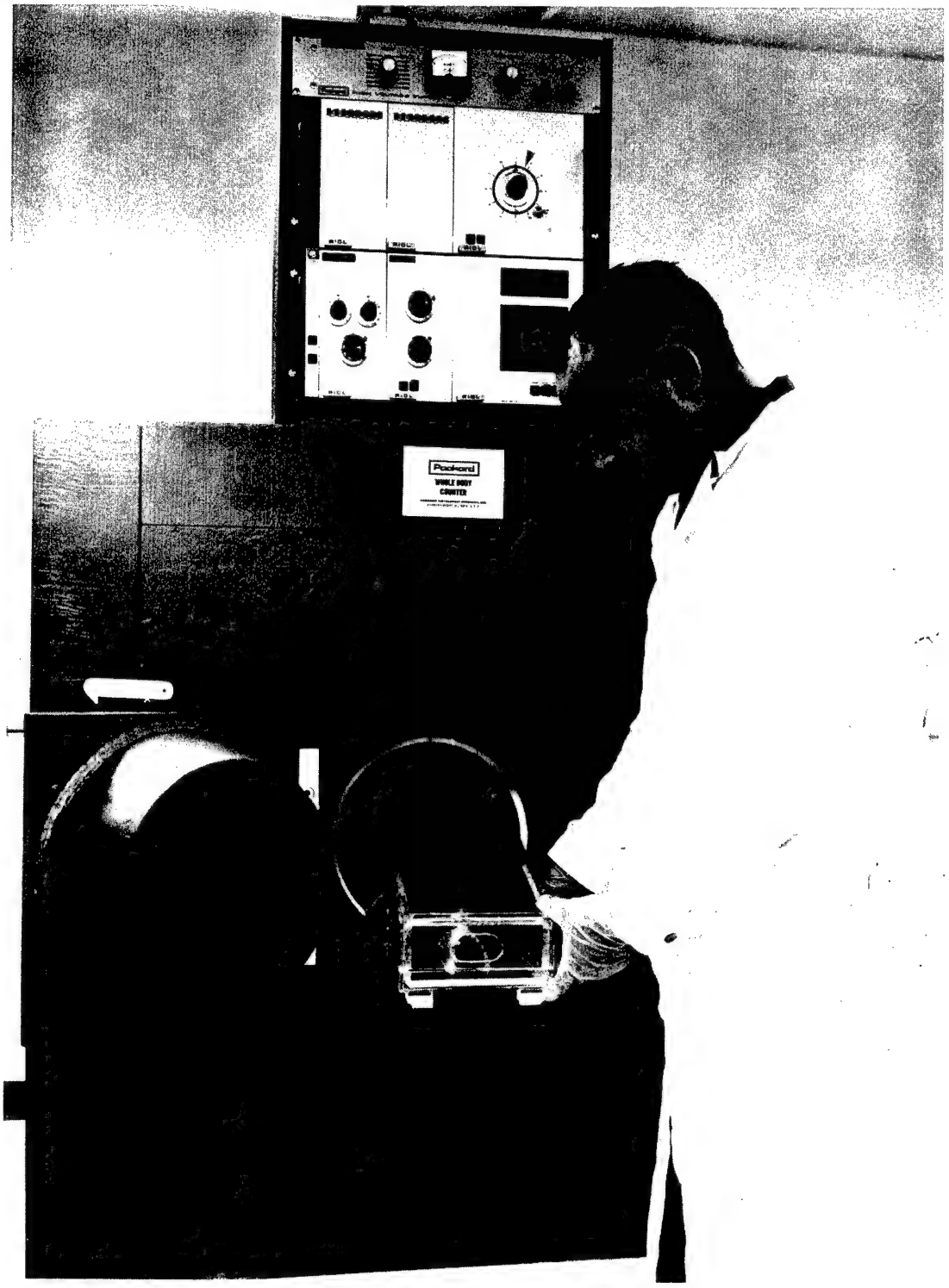


Figure 2: Dog being counted in the large volume, liquid scintillation counter. Counting chamber is 36 inches long and has a 12-inch diameter. The shield is constructed of special low-activity steel and is 6 inches thick.

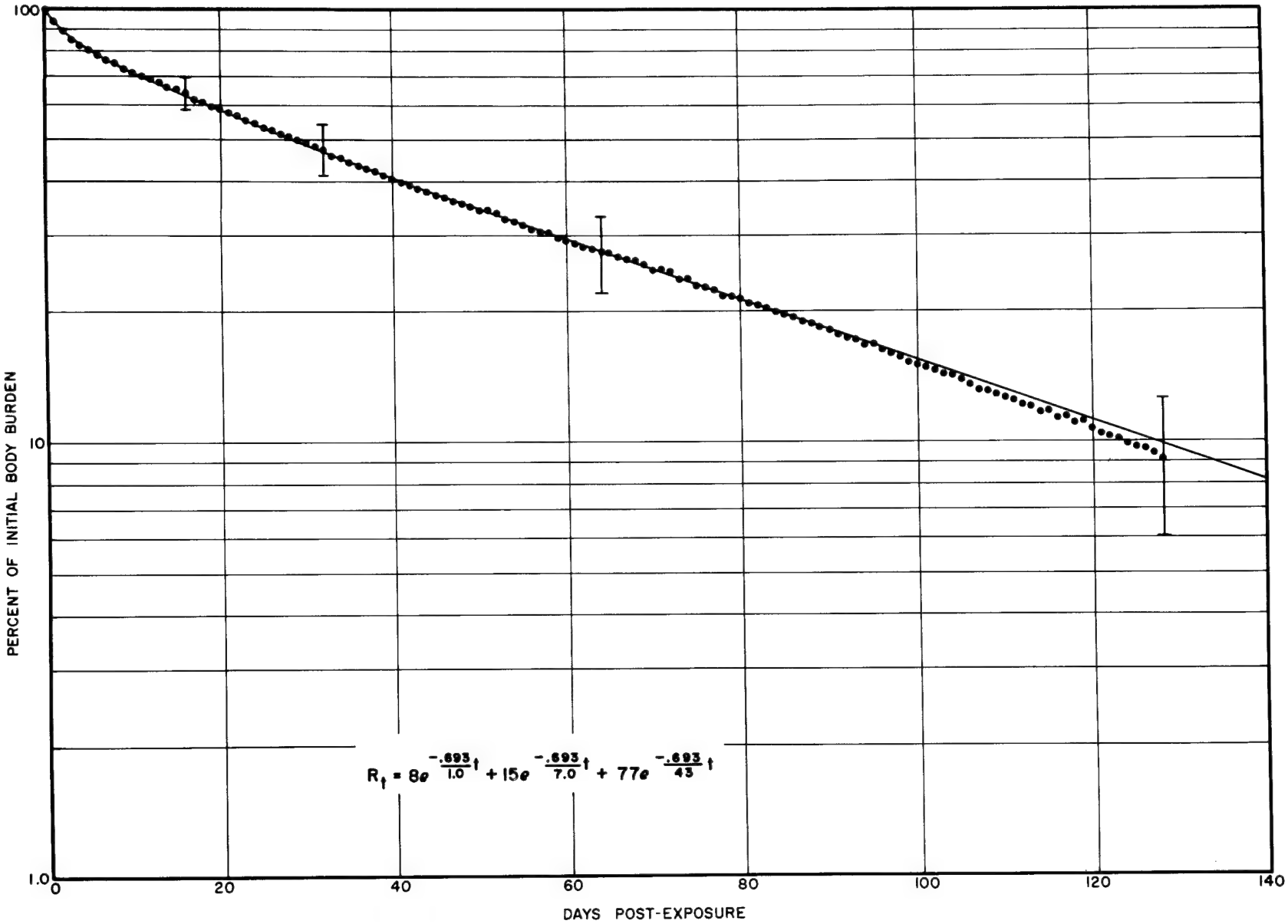


Figure 3: Whole body retention of Cs-137 following a single inhalation exposure. The points represent a mean of 20 dogs through day 16, 18 dogs through day 32, 15 dogs through day 64 and 11 dogs through day 128. The vertical lines at 16, 32, 64 and 128 days represent ±1 standard deviation.

closely by examination of the individual curves presented in Figures 4, 5, 6 and 7 for the 8 dogs that were sacrificed at 256 or 366 days post-exposure.

The first 2 dogs, 01-065 and 02-065, have values of T_b = 32 and 37 days, respectively, for the long-term component and no break in the retention pattern is indicated. The 6 remaining dogs, however, all demonstrate a change to a faster excretory rate during the course of the experiment. An idea of the magnitude of this change can be obtained by comparing the dotted line, which is a straight line extension of the rate prevailing just before the break, with the solid line drawn through the actual data points. A summary of the values of T_b just before and after the break is given in Table 1. The mean value of T_b is 42 days just prior to the break and is 36 days after the break.

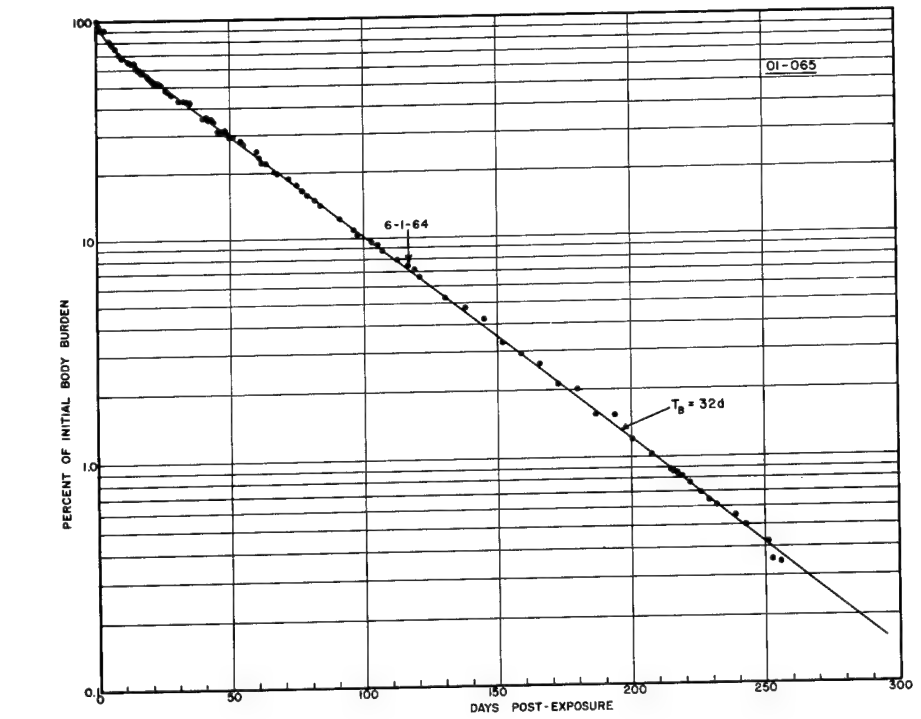
The second group of dogs that were exposed following completion of the 25-dog experiment are currently at about 100 days post-exposure and the whole body counting will be continued until their sacrifice at 365 days post-exposure. Curves drawn from the data obtained to date yield values of T_b equal to 21, 26, 28, 35 and 38 days for the long-term component for the 5 dogs. The curve drawn through the arithmetic mean values of these data also can be expressed as a 3-component exponential:

$$R_t = 20e^{-.770t} + 14e^{-.069t} + 66e^{-.023t}$$

The three exponents represent half-lives of 0.9, 10 and 30 days.

DISCUSSION

The time at which the increased excretion of Cs-137 occurred is given in Table 1 as days post-exposure and also as the number of days after June 1, 1964. The wide range of days post-exposure at which the change occurred indicates that the change was not a function of the exposure per se. Instead, it appears to be associated with a calendar date near June 1, 1964. As mentioned under Methods, the diet for the entire dog colony was changed at about this time (exact date not recorded). Since Mraz, et al.[2] and Richmond, et al.[3] have shown that an increase in dietary potassium can enhance the excretion of Cs-137, the potassium levels in the old



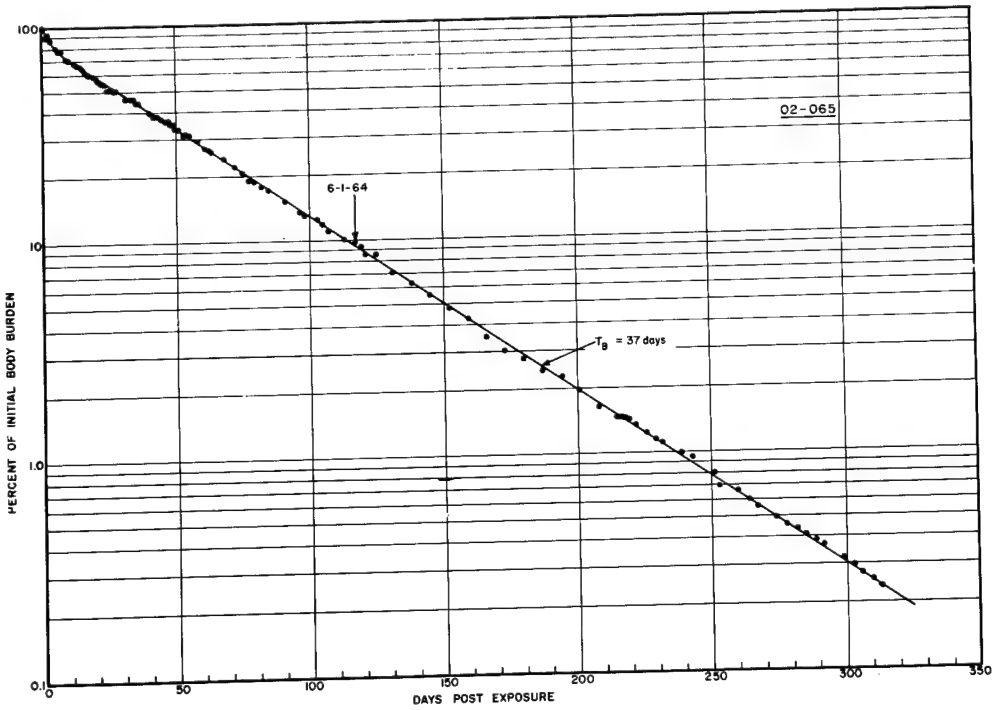
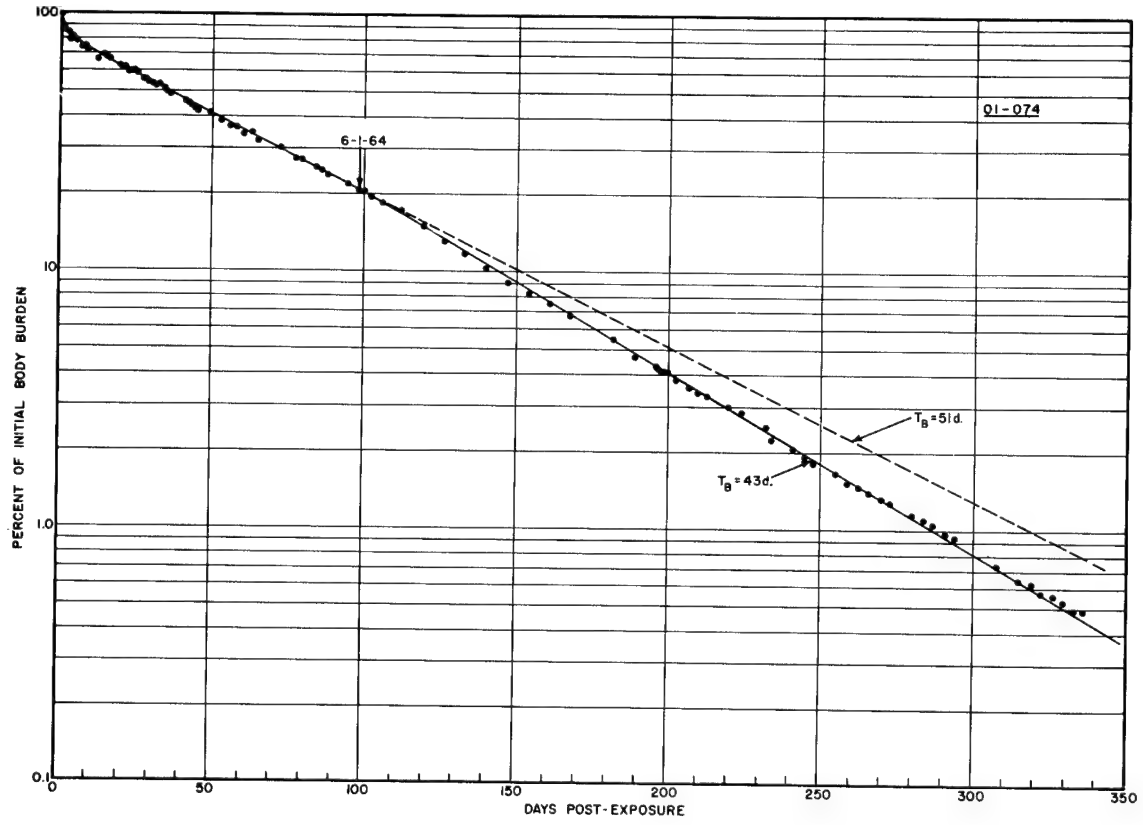


Figure 4: The whole body retention of inhaled Cs-137 for dogs 01-065 and 02-065.



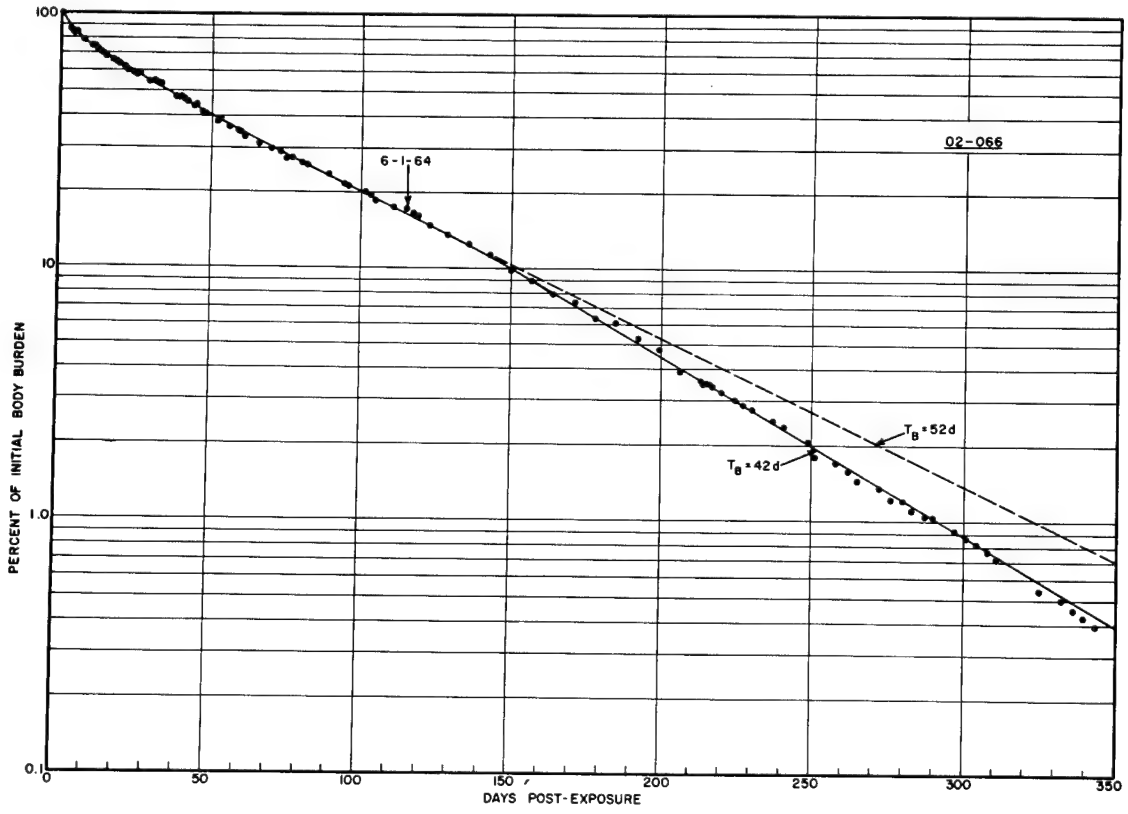
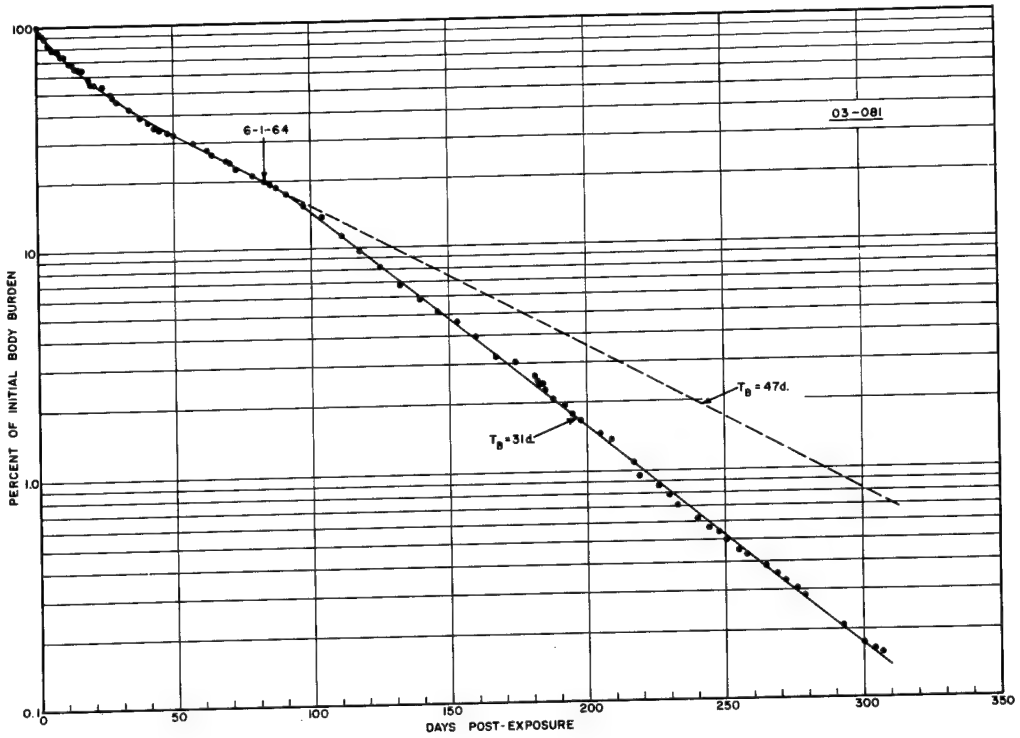


Figure 5: The whole body retention of inhaled Cs-137 for dogs 02-066 and 01-074.



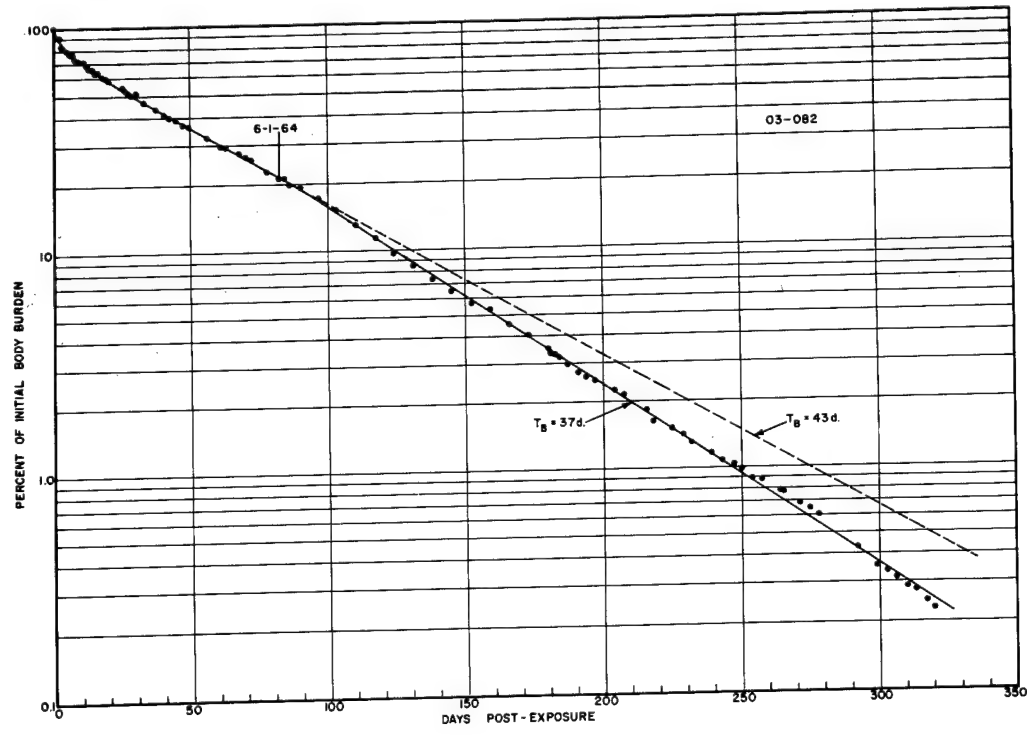


Figure 6: The whole body retention of inhaled Cs-137 for dogs 03-081 and 03-082.

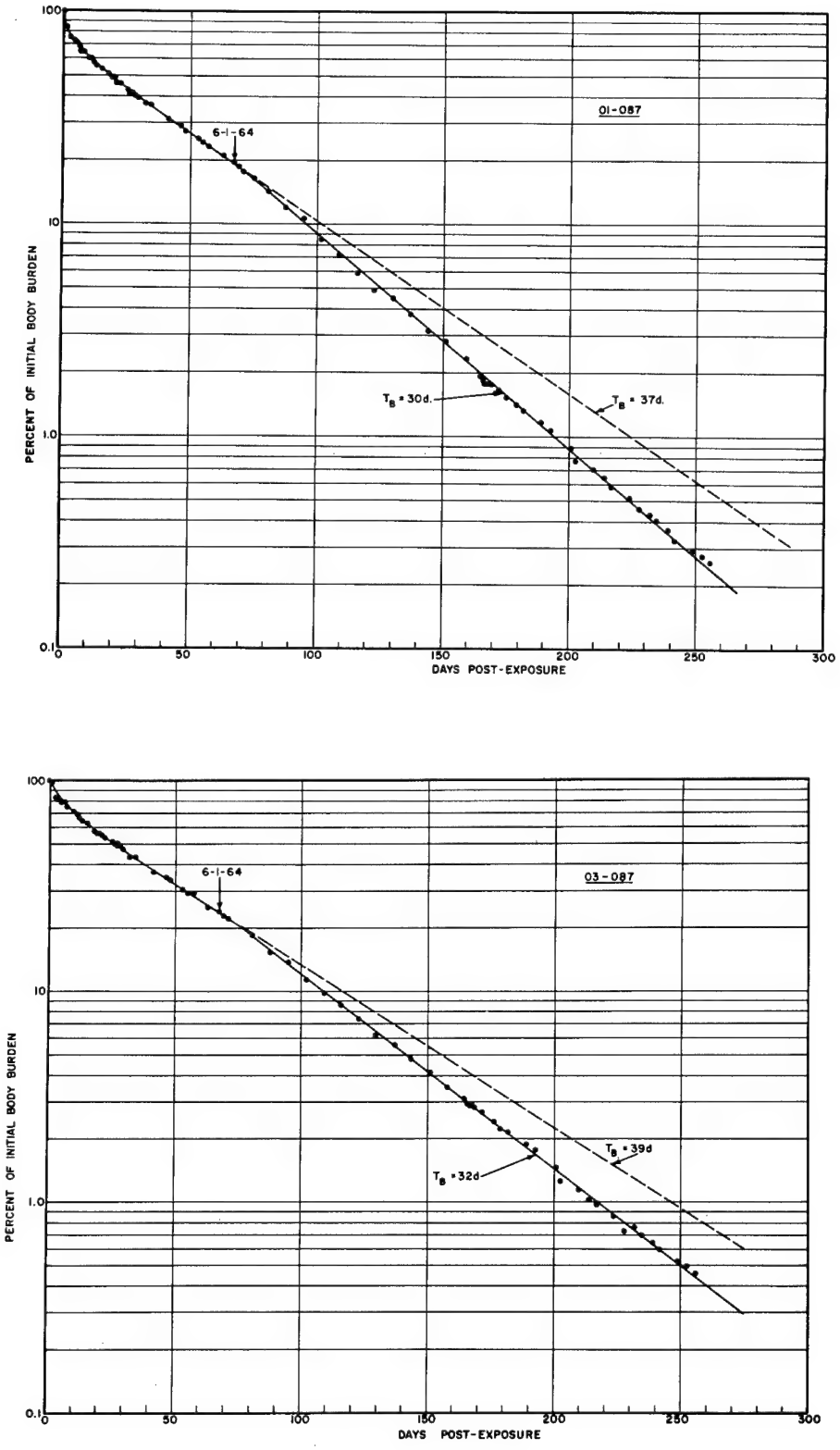


Figure 7: The whole body retention of inhaled Cs-137 for dogs 01-087 and 03-087.

TABLE 1

Values of T_b Observed Before and After the Break in the Retention Curve

Dog No.	Sex	Age	T_b Before 6/1/64	T_b After 6/1/64	Time Break Occurred: Days Post-Exposure	Time Break Occurred: Days After 6/1/64
01-065	F	34 mo.	32 d.	32 d.	-	-
02-065	F	27	37	37	-	-
02-066	M	17	52	42	140	25
01-074	M	20	51	43	104	6
03-081	F	22	47	31	91	8
03-082	F	22	43	37	93	11
01-087	M	21	37	30	73	6
03-087	M	21	39	32	79	12
			$\bar{x} = 42$	$\bar{x} = 36$		

and new diets were examined. The Wayne Dog Food used exclusively for the original diet normally contains 0.73 per cent potassium[4]. In order for the new diet to have a significantly higher potassium level, the ground beef or the Vionate® supplement would have to contain much more than 0.73 per cent potassium. Since heart, liver and muscle tissue from cattle contain only 0.3 to 0.5 per cent potassium[5] and since potassium is not listed as being one of the 9 minerals in Vionate®, the possibility of an increased potassium level with the new diet can be ruled out.

By coincidence, the taking of weekly dog weights was begun at approximately the same time that the diet was changed. Figure 8 shows the weekly weight of each dog in relation to its weight at the time of exposure --- day 121 corresponds to June 1, 1964 and at this time 6 of the 8 dogs weighed less than they did at exposure 3 to 4 months earlier. From June 1, 1964 until sacrifice, 1 dog had a 4 per cent weight increase and the other 7 had increases of 12 to 30 per cent. After the diet change, the caretakers also noted improved coats on these dogs. Both of these observations indicate that the dogs general state of health was improved following the diet change and this was probably responsible for the change in the Cs - 137 excretory rate. It is not clear why there was no change observed in the excretory rate for dogs 01-065 and 02-065 even though they both exhibited substantial weight gains after the diet change.

The values of T_b obtained here can be compared with those observed by other investigators following intravenous injection. Norris et al.[6] reported a value of T_b = 25 - 30 days based on data from 8 dogs obtained during the first 130 days following injection. Richmond followed 4 dogs for a year and obtained data whose mean values could be fitted by the following 3-component exponential equation:

$$R_t = 14e^{-.630t} + 56e^{-.025t} + 30e^{-.015t}$$

From this equation the value of T_b in the longest-term component is about 43 days. However, if this curve is only plotted from 0 - 100 days, i.e. the approximate range covered by the data in Figure 3 and in the Norris data, the longest-term component can be fitted by using a value of T_b = 33

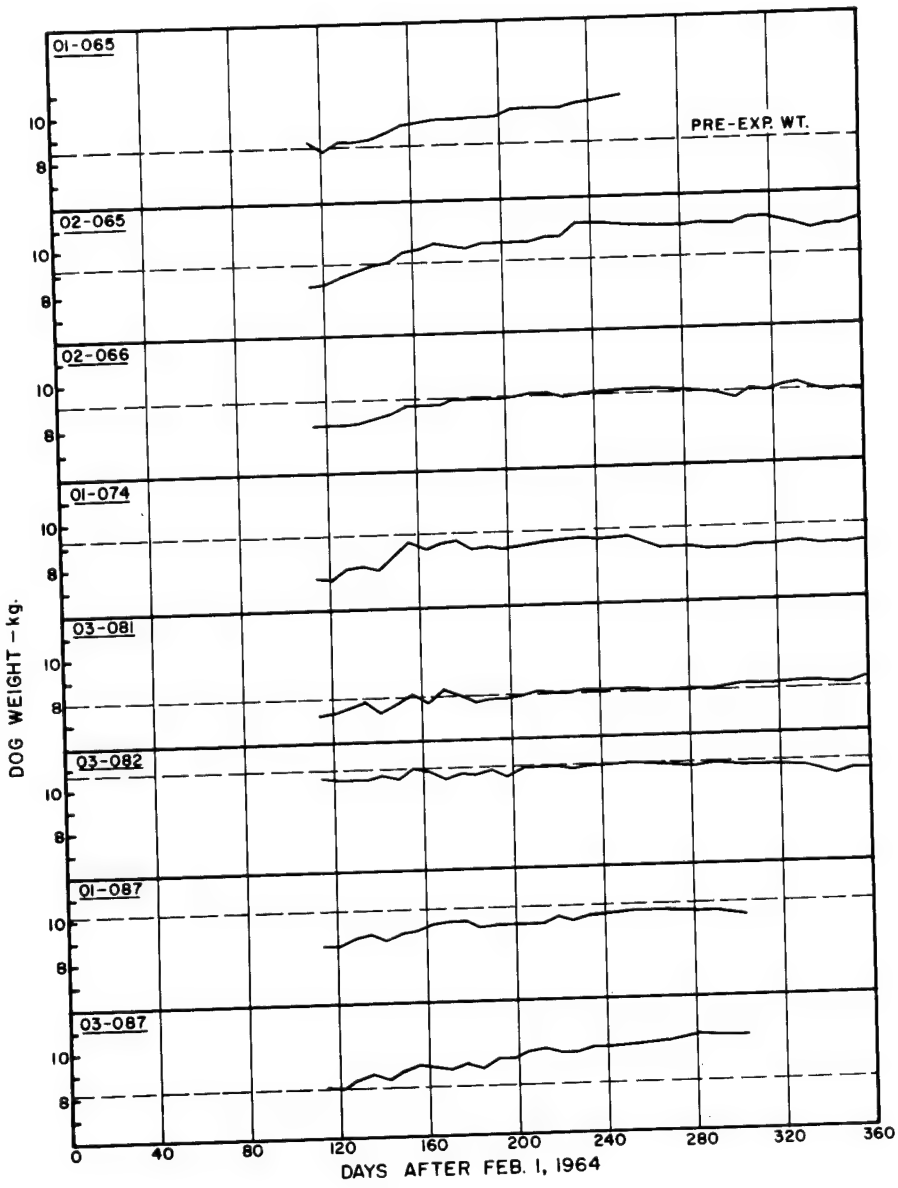


Figure 8: Weekly dog weights in relation to their pre-exposure weights. All exposures occurred near Feb. 1, 1964 but weekly weights were not recorded until May 25, 1964.

days. The Norris value and the Richmond value at 100 days are significantly lower than the value of T_b seen with the present data just prior to the break in the retention curve. The mean T_b observed after the break, 36 days, is more in line with the values of T_b reported by the investigators mentioned above.

It is interesting to note that once the break occurred, no other changes in the excretory rate were seen. This is in contrast to the data of Richmond in which the influence of the 43-day component became more pronounced at times greater than 150 days after injection.

One of the reasons for performing this experiment was to obtain normal distribution and excretion patterns for use in planning future longevity experiments with high levels of Cs-137. These future experiments will differ from the 25-dog experiment described above in several ways:

A. The dogs will all be of the same age and younger than those used here.

B. Their diet will be of constant composition throughout the experiment.

C. Most of the dogs will spend only the first 30 days post-exposure in metabolism cages. The remainder of their life will be spent in kennels with outdoor runs.

The main reason for the second experiment with 5 dogs was to determine the importance of the above differences. Although these dogs are not as young as those to be used in the future (13±0.5 months), their age at exposure was uniform and much less than some of the dogs used in the first experiment. In spite of the uniformity of age and environmental conditions, the individual retention patterns observed to date vary considerably. The value for the long-term component of the curve fitted to the mean data for these 5 dogs between 50 and 100 days post-exposure is 30 days, a value in excellent agreement with the data of Richmond and the early data of Norris. It will be interesting to determine whether the value of T_b for the long-term component becomes longer as observed with the Richmond data.

More recent data by Norris et al.[8] have shown an apparent dependence of T_b on the age of the dog. Mean values of T_b equal to 16 days for 5-month old dogs, 20 days for 13-month old dogs and 37 days for dogs that were 4 - 5 years of age at injection have been observed. Since the experiments involved high levels of Cs-137 it is not clear whether this age effect is the result of a varying response to radiation with age or is actually a variation in Cs-137 retention with age. In the present experiments tracer levels were used and no correlation between variability in metabolism of Cs-137 and age was observed.

CONCLUSIONS

These data emphasize the need for maintaining constant environmental conditions during distribution and excretion experiments. The agreement between the data obtained following inhalation (particularly for the second experiment) and the data reported by others following intravenous injection substantiates what one would expect: for materials that are very soluble in body fluids, the route of administration is not critical. The considerable variation among the retention patterns of the 5 dogs in the second experiment emphasizes the necessity for determining the retention pattern in each dog in order to calculate the integrated dose occurring between exposure and death.

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SURVIVAL OF RATS FOLLOWING INTRAPERITONEAL INJECTION OF ACUTE DOSES OF Sr-90 AND Cs-137, SINGLY AND IN MIXTURES

by
R. Lie and R. G. Thomas

In keeping with the goals of the 1013 program it was decided to study the effects of a mixture of two fission products introduced into rats. For an animal to deposit a certain quantity of radioactive material in the lung by inhalation, approximately one thousand times this amount must be present in the aerosol generator, using exposure apparatus currently available. To obtain a whole body concentration great enough to cause acute radiation effect considerable personnel hazard may be involved, and certain adjustments to exposure equipment must be made to accommodate additional shielding material. Because of these reasons it was decided to inject high levels of various mixtures of two isotopes intravenously. Since Cs-137 and Sr-90 had been studied individually they were chosen for the first mixed fission product experiment. The intraperitoneal route of injection was chosen for its ease and rapidity.

EXPERIMENTAL DESIGN

Holtzman strain albino rats were used. Average weights at injection were 151 grams (male) and 173 grams (female). The injection regime is on the following page.

Group	Dose Cs-137	Dose Sr-90	No. Animals	Sex
I a	0	5 mc/kg	20	♂
b	0	5 mc/kg	20	♀
II a	25 mc/kg	0	20	♂
b	25 mc/kg	0	20	♀
III a	25 mc/kg	2.5 mc/kg	20	♂
b	25 mc/kg	2.5 mc/kg	20	♀
IV a	12.5 mc/kg	5 mc/kg	20	♂
b	12.5 mc/kg	5 mc/kg	20	♀
V a	12.5 mc/kg	2.5 mc/kg	20	♂
b	12.5 mc/kg	2.5 mc/kg	20	♀
VI a	0	0	40	♂
b	0	0	40	♀
VII a	0	0	40	♂
b	0	0	40	♀

All animals were housed by pairs in standard small animal cages. Groups I through VI were housed on the racks in a random manner so that the external doses received by any pair of animals were no more magnified for one experimental group than for another. Levels in the animals were high enough for the rats to serve as external radiation sources. Group VII was maintained in an area apart from all other groups, thereby assuring a true control group for longevity purposes.

Excreta were collected from all animal pairs (Groups I through VI) for several days and thereafter from Groups I through V. Samples were collected in aluminum foil trays constructed to fit beneath the individual cages. Combined urine and feces were collected and analyzed together for gross radioactive content. One animal from each cage was counted in a plastic container on the day following injection. The second of each animal pair was counted during the subsequent week. Upon death, each animal was weighed and counted. Several from each experimental group were frozen when discovered dead, and will be dissected at a later time to determine tissue distribution of the isotopes.

PRELIMINARY RESULTS

The first death due to radiation occurred in Group III on the fifth day following isotope injection. All animals from that group had succumbed by day 12; from Group IV by day 13; and from Group V by day 21. A death occurred in Group I on the ninth day, but the incidence rate was much less than in Groups III through V, the last animal dying on day 32. The first death in Group II was on day 12 with the majority of these animals having died by the fourtieth day. Four of these animals are still surviving 138 days following injection. No significant difference between males and females with respect to longevity has been noted.

The general physical appearance of all animals at death has been one of emaciation. Lack of appetite was very apparent in Groups III through V within a very few days following injection, but was somewhat less evident in Groups I and II. Weight loss was marked in all experimental animals. Expressed as percent of initial weight, the average loss was 35, 34, and 33 percent in Groups III, IV and V respectively, and 40% in Groups I and II. This difference was presumably due to greater longevity in the latter two groups.

Whole body counting data have been preliminarily analyzed for Groups II through V; no sex or group differences in isotope retention are immediately apparent. Additional analyses will be performed on data from whole body and excreta counting, and from film badges which were placed on the exterior sides of all cages of animals in Groups I through VI. These were placed in order to measure the external radiation dose.

THE HEMATOLOGIC RESPONSE IN RATS TO INTRAPERITONEAL INJECTION OF MIXED FISSION PRODUCTS - SR-90 AND CS-137

by

R. K. Jones

PURPOSE AND METHODS

This pilot experiment was designed to investigate comparative biological effects of intraperitoneally administered Sr-90, Cs-137 and mixtures of these two fission products. Although the death distribution resulting from exposure to the single or mixed isotopes was the primary goal, a limited number of animals were similarly injected in order to study the hematologic effects. The primary hematologic responses of interest were the synergistic effects of strontium and cesium when administered as a mixture. Unfortunately the doses resulted in relatively early demise of the experimental rats, and therefore only acute effects could be ascertained.

Equal numbers of male and female Holtzman rats were divided into five experimental groups. Each group consisted of 10 animals which were sampled periodically for hematologic changes (see Table 1 - Exposure Schedule). Additional groups were used as controls and sampled periodically. Some of these were maintained in the room with the experimental animals and others at a remote location where they received no irradiation. All animals weighed approximately 200 grams at injection. Sampling was performed at 40 hours and 5, 9, 14, 21, 28 and 35 days following injection.

PROGRESS REPORT

Since the hematologic values for both "inside" and "outside" controls were similar, these were combined and treated as one control population

Table 1

ANIMALS EXPOSED FOR HEMATOLOGICAL EVALUATION

Group Number	Sr^{90} mc/kg	Cs^{137} mc/kg	Number of Animals	Number of Survivors at 35 Days	Mean Day of Death for Nonsurvivors Dying before 35 Days
I	5	0	10	0	20
II	0	25	10	3	26
III	2.5	25	10	0	9
IV	5	12.5	10	0	9
V	2.5	12.5	10	0	11
VI	inside* control	inside* control	10	10	-
VII	outside** control	outside** control	10	10	-

*Rats housed in the same room as injected subjects.

**Rats housed in separate animal room.

(Figure 1). All experimental animals showed a profound fall in leucocytes within the first 40 hours to a level ranging from approximately 35 to 40% of pre - exposure values. A progressive depression continued until the ninth day post-exposure when death had occurred in all animals that received a mixture of strontium and cesium (Groups III, IV, V). An abortive recovery was seen in the two surviving groups on the 14th day with a subsequent depression by the 21st day post - exposure. At this time no survivors were present in Group I. The surviving cesium animals revealed a slow recovery to the final day of observation (35 days post-exposure). The slope of the initial and more delayed fall in total leucocyte count was roughly similar for all exposure groups.

Total lymphocyte count revealed a marked depression by 40 hours and showed a secondary abortive rise at five days post-exposure (Figure 2). The magnitude of depression was slightly different between groups, being most profound in animals injected with cesium and less severe in animals injected with strontium. This type of response would be predicted on the basis of the type of radiation produced by these two isotopes, illustrating the differences between beta and gamma emitters.

The total neutrophil count showed an elevation at 40 hours followed by a prompt depression which was minimal at nine days (Figure 3). The magnitude of depression in each experimental group was roughly the inverse of lymphocytes; strontium-injected rats showed the greatest depression and cesium appeared somewhat less damaging. This response also would be expected on the basis of difference in the retention sites of the two isotopes, strontium being mostly deposited in bone adjacent to marrow whereas cesium seeks soft tissues predominantly where it acts more as a source of whole body radiation.

With the exception of animals receiving five microcuries of strontium, either alone or in combination with cesium, depression of the hematocrit, hemoglobin and red blood count was less marked than that seen in white corpuscular elements. For purposes of illustration only the hematocrit is included (Figure 4). However, in the two groups of animals receiving the high level strontium dose, depression of the erythroid elements was

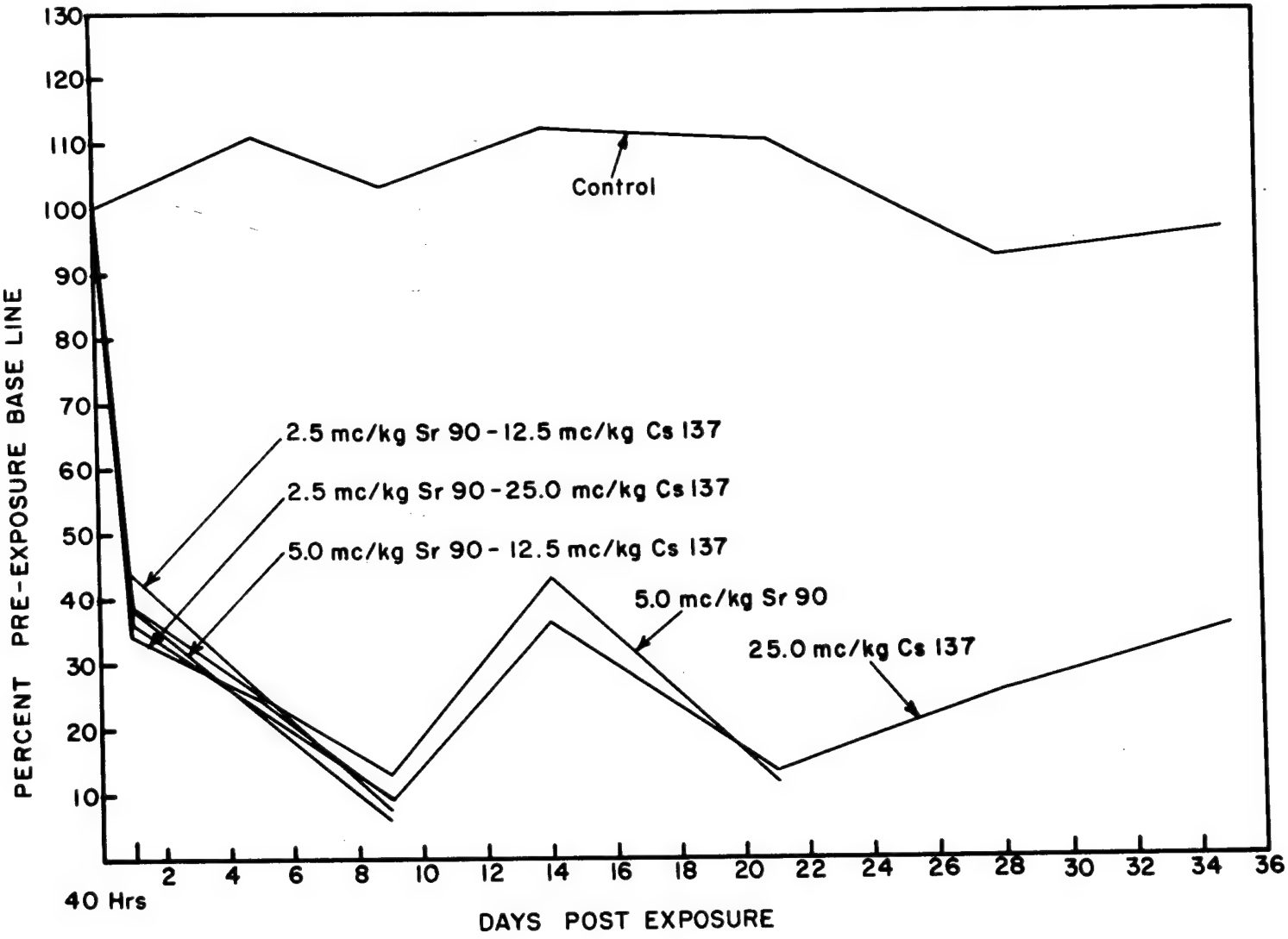

Figure 1: Total white blood cell response expressed in mean per cent of pre-exposure baseline values.

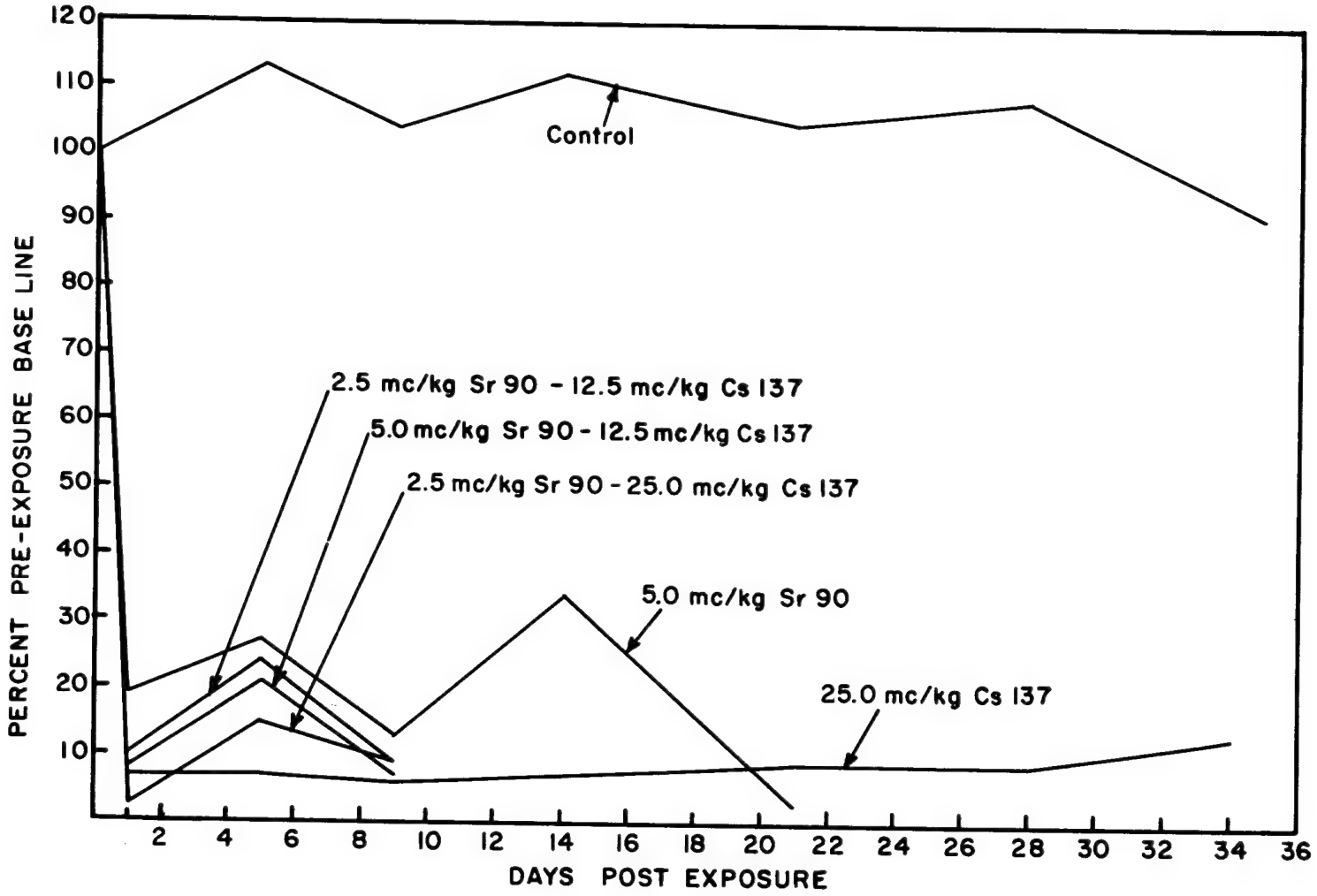

Figure 2: Lymphocytic response (absolute numbers) expressed in mean per cent of pre-exposure baseline values.

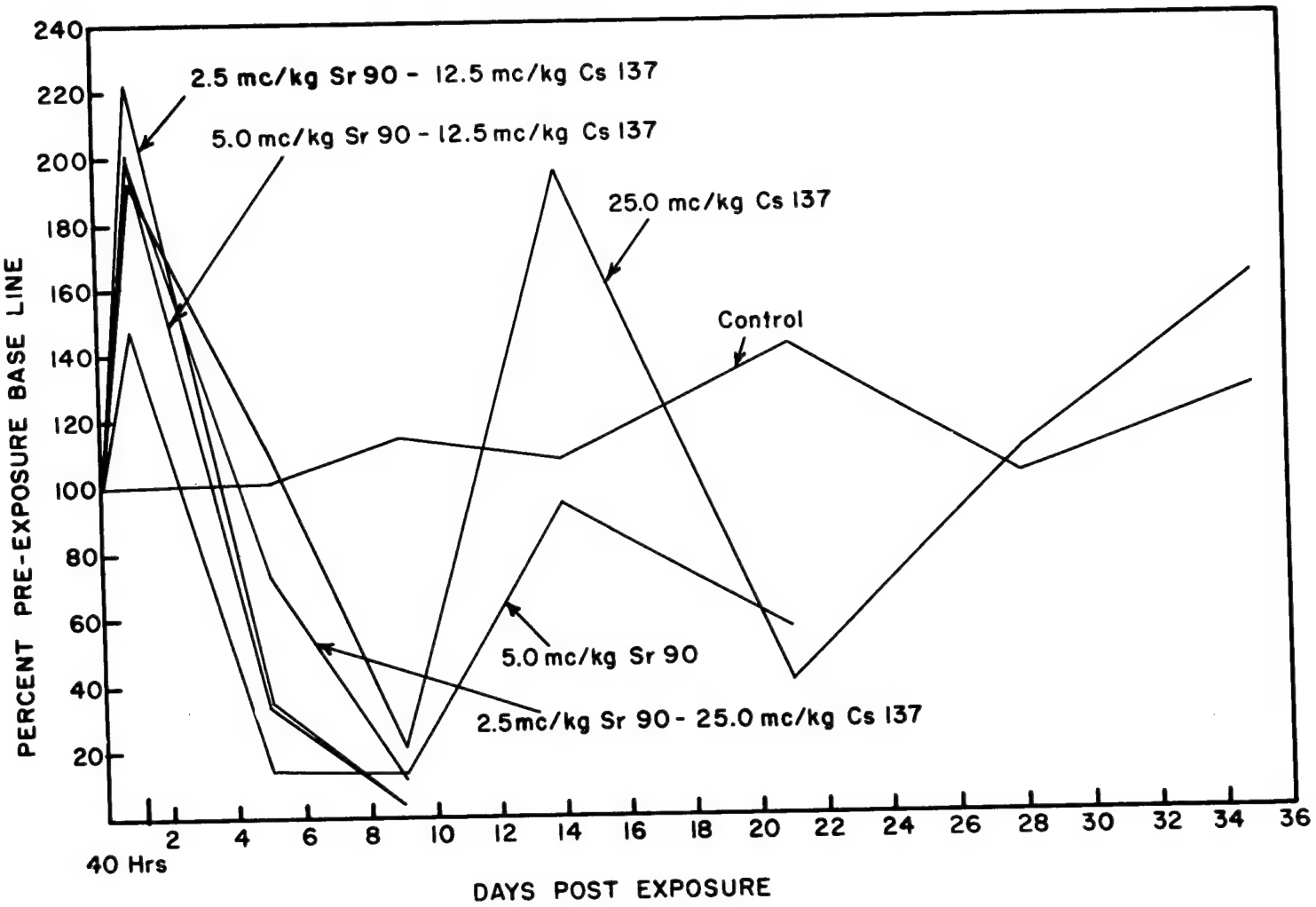

Figure 3: Granulocytic response (absolute numbers) expressed in mean per cent of pre-exposure baseline values.

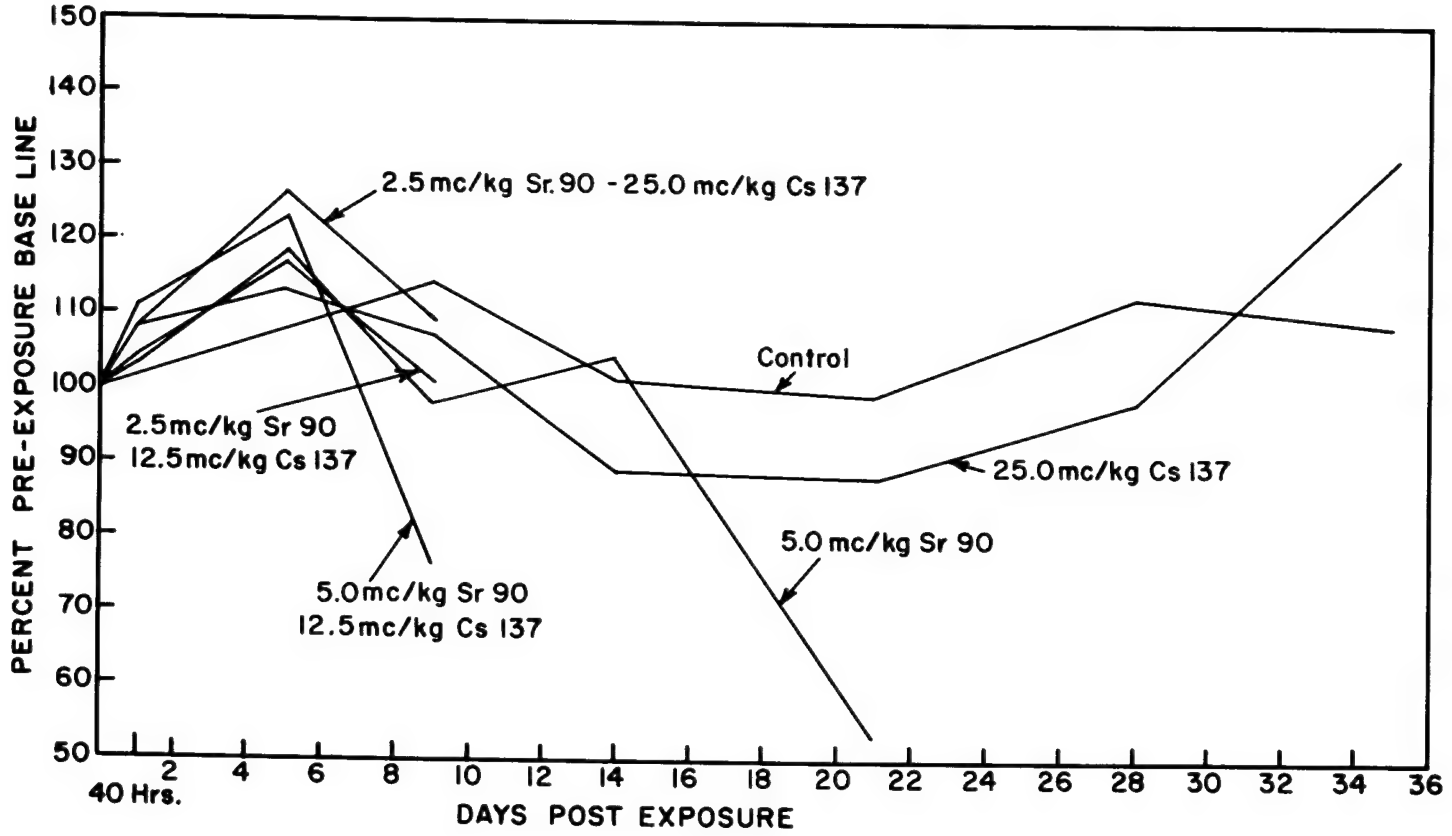

Figure 4: Packed erythrocyte volume expressed in mean per cent of pre-exposure baseline values.

considerably more severe.

It appears that predictable differences occurred in hematopoietic damage induced by Sr-90 and Cs-137. Cs-137, being a generally distributed gamma emitter, produced a more detrimental effect on circulating lymphocytes whereas Sr-90, being a beta emitter selectively incorporated into bone, manifested a greater effect on granulocytes and erythrocytes. It is also apparent from this pilot experiment that considerably lower doses of Sr-90 and Cs-137, both singly and in combination, must be administered in subsequent studies, planned for the near future, to delineate any synergistic effects.

THE EFFECT OF INHALATION OF FISSION PRODUCTS AND AGING ON THE BEAGLE ELECTROCARDIOGRAM

by

H. C. Redman and J. E. Weir

INTRODUCTION

Electrocardiograms are being obtained on the beagles in the colony prior to and at suitable intervals after exposure to ionizing radiation. These records will help determine possible effects of inhaling fission products on the cardiovascular system. Most information available on the electrocardiogram of the dog is based on limited observations made on mixed breeds of varying age, sex, size, and state of health[1-4], but some data are available on a limited number of beagles[5]. Since this study reports findings on only a small number of recordings made on dogs of widely varying age, it is not now possible to establish a baseline with which variations with respect to ageing can be ascertained. However, this will be forthcoming subsequently from studies of tracings obtained periodically throughout the life span of control animals. Pre-exposure tracings will be obtained on all animals which are twelve months old, and annually thereafter on all control animals.

METHODS

Electrocardiograms are recorded as a part of a general physical examination which is described in another section of this report. To obviate the marked effects of position on the electrocardiogram in dogs the tracings are obtained with the beagle in a normal standing position, but constrained by a canvas sling to help prevent excessive movement of the unanesthetized and unmedicated animals that have been adapted by suitable training to remain quiet. Electrodes are applied over electrode paste to unshaven skin. Recordings are obtained in an isolated room to help avoid

exciting the animal. The leads recorded in this study are presented in Table 1, and are those considered to be of greatest diagnostic value by Detweiler[1-3] and Lanneck[4]. Figure 1, after the method of Lanneck[4], illustrates the technique used in measuring duration and amplitude on the EKG.

RESULTS

Presented below are data obtained from 39 dogs which were raised, in kennels located at the Laboratory, in accordance with previously published procedures[6]. Individual animals were in satisfactory health at the time of study since no significant abnormalities were detected during physical examination.

The only arrhythmia noted was sinus arrhythmia which is normal for a resting dog[2].

Table 2 shows the mean and standard deviations for the amplitudes obtained. The mean and standard deviations of the duration values are presented in Table 3.

EKG LEADS

I. Screen lead (unipolar): CR_6L (chest right 6th intercostal, lower) the exploring electrode is connected to the bottom of the sixth intercostal space. The indifferent electrode is connected to the right foreleg.

II. Standard

A. Bipolar Leads

Lead I – the electrodes are connected to proximal parts of the right foreleg and left foreleg (posterior olecranon), where the subcutis is loose, the left foreleg being relatively positive, the right foreleg being relatively negative.

Lead II – the electrodes are connected to the same region of the right foreleg, the other connected just distal to the anterior surface of the left femor where the subcutis is loose. The right foreleg is negative and the left leg relatively positive.

Lead III – the electrodes are connected to the left foreleg and left leg, the left foreleg being relatively negative and the left leg relatively positive.

B. Augmented Unipolar Leads

aVR – the electrode records the potential from the right foreleg.

aVL – the electrode records the potential from the left foreleg.

aVF – the electrode records the potential from the left leg.

C. Unipolar Precordial Leads

CR_6L – the exploring electrode is connected at the 6th lower left intercostal space at the bottom (near the edge of the sternum) at the apex of the left ventricle. The indifferent one on the right foreleg.

CR_6U – the exploring electrode is located at the 6th left intercostal space at the costochondral junction over the free wall of the left ventricles. The indifferent one on the right foreleg.

CR_5 – the exploring electrode is at the 5th right intercostal space near the edge of the sternum over the free wall of the right ventricle. The indifferent one on the right foreleg.

CR_6L, CR_6U, and CR_5 as established by Lannek against Wilson's central terminal (V).

TABLE 1

MEASUREMENTS of EKG DURATIONS and AMPLITUDES

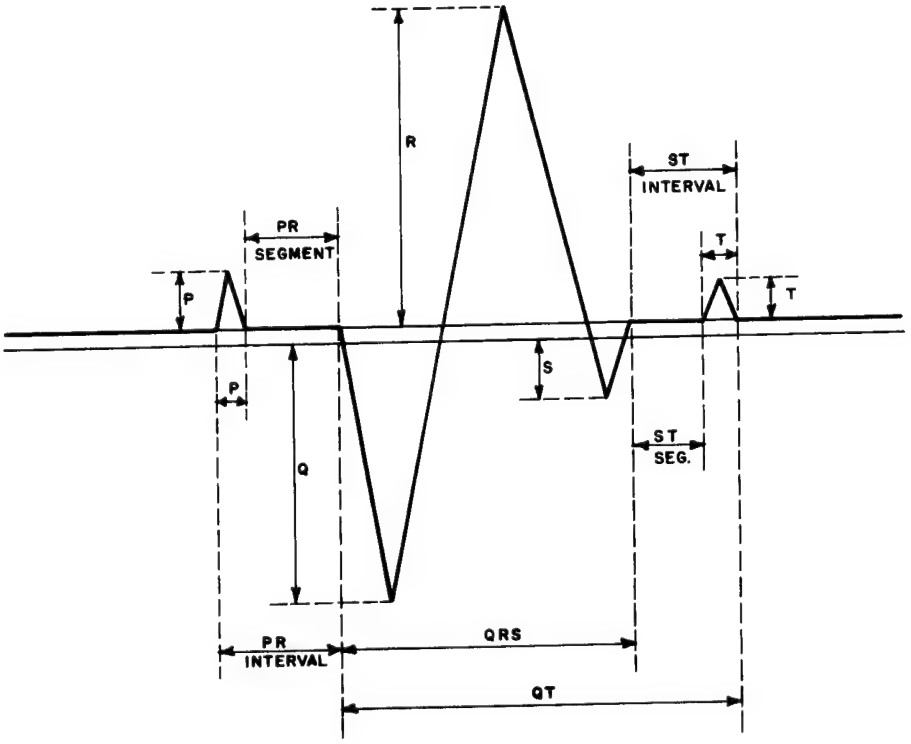

FIGURE 1

TABLE 2

EKG

Amplitude (mv)

Lead		P	Q	R	S	T
I	Mean	0.102	-0.277	0.544	-0.244	+0.149 -0.122
	Standard Deviation	0.0309	0.6199	0.2793	0.5340	0.0918 0.2624
II	Mean	0.166	-0.195	1.699	-0.382	+0.230 -0.133
	Standard Deviation	0.0672	1.354	0.6980	0.8304	0.2145 0.2977
III	Mean	0.098	-0.219	1.418	-0.391	+0.172 -0.157
	Standard Deviation	0.0345	0.4817	0.5863	0.8588	0.1574 0.3349
AVR	Mean	+0.082 -0.142	-0.903	0.386	-0.983	+0.131 -0.187
	Standard Deviation	0.0589 0.1617	1.928	0.3568	2.1020	0.0915 0.4149
AVL	Mean	+0.079 -0.080	-0.577	0.412	-0.679	+0.158 -0.141
	Standard Deviation	0.0332 0.0169	1.2610	0.2778	1.4390	0.1315 0.3082
AVF	Mean	+0.141 -0.200	-0.163	1.410	-0.385	+0.190 -0.181
	Standard Deviation	0.0524 0.5545	0.3581	0.6232	0.8339	0.1931 0.3922
CR_6L	Mean	0.122	-0.115	1.38	-0.511	+0.233 -0.078
	Standard Deviation	0.3493	0.2524	0.6917	1.0900	0.167 0.1723
CR_6U	Mean	0.111	-0.132	1.198	-0.380	+0.181 -0.105
	Standard Deviation	0.0382	0.2955	0.5320	0.7991	0.1517 0.2280
CR_5	Mean	+0.068 -0.111	-0.297	0.993	-0.377	+0.235 -0.116
	Standard Deviation	0.0218 0.2746	0.8142	0.4998	0.8510	0.1882 0.4118

TABLE 3

EKG

Duration (Seconds)

INTERVAL		I	II	III	AVR	AVL	AVF	CR_6L	CR_6U	CR_5
P	Mean	0.036	0.035	0.035	0.035	0.032	0.034	0.035	0.0355	0.033
	Standard Deviation	0.0076	0.0053	0.0061	0.0059	0.0054	0.0038	0.0051	0.0061	0.0057
PR Segment	Mean	0.056	0.053	0.053	0.054	0.056	0.053	0.053	0.053	0.053
	Standard Deviation	0.0099	0.0095	0.0108	0.0092	0.0102	0.0103	0.0103	0.0109	0.0120
PR Interval	Mean	0.092	0.089	0.086	0.088	0.088	0.084	0.088	0.088	0.086
	Standard Deviation	0.0110	0.0110	0.0107	0.0115	0.0122	0.0102	0.0113	0.0110	0.0122
QRS	Mean	0.036	0.038	0.038	0.038	0.038	0.038	0.038	0.038	0.037
	Standard Deviation	0.0048	0.0045	0.0055	0.0054	0.0040	0.0041	0.0036	0.0059	0.0042
QT	Mean	0.184	0.190	0.190	0.188	0.188	0.189	0.192	0.191	0.188
	Standard Deviation	0.0152	0.0175	0.0165	0.0172	0.0177	0.0181	0.0182	0.0172	0.0181
T	Mean	0.034	0.037	0.035	0.035	0.034	0.033	0.037	0.033	0.035
	Standard Deviation	0.0134	0.0148	0.0127	0.0169	0.0099	0.0103	0.0115	0.0083	0.0301
ST Interval	Mean	0.145	0.152	0.152	0.150	0.151	0.152	0.154	0.154	0.150
	Standard Deviations	0.0260	0.0171	0.0170	0.0168	0.0154	0.0157	0.0171	0.0167	0.0170
ST Segment	Mean	0.114	0.115	0.116	0.116	0.117	0.120	0.116	0.120	0.117
	Standard Deviations	0.0194	0.0210	0.0198	0.0191	0.0185	0.0195	0.0183	0.0195	0.0184

CONCLUSIONS

For the beagle of one year of age, it is probable that these data represent a normal range of values. It is also probable that any findings differing markedly from these data are indicative of a significant abnormality.

EKG LEADS

I. Screen lead (unipolar): CR_6L (chest right 6th intercostal, lower) the exploring electrode is connected to the bottom of the sixth intercostal space. The indifferent electrode is connected to the right foreleg.

II. Standard

A. Bipolar Leads

Lead I - the electrodes are connected to proximal parts of the right foreleg and left foreleg (posterior olecranon), where the subcutis is loose, the left foreleg being relatively positive, the right foreleg being relatively negative.

Lead II - the electrodes are connected to the same region of the right foreleg, the other connected just distal to the anterior surface of the left femur where the subcutis is loose, The right foreleg is negative and the left leg relatively positive.

Lead III - the electrodes are connected to the left foreleg and left leg, the left foreleg being relatively negative and the left leg relatively positive.

B. Augmented Unipolar Leads

aVR - the electrode records the potential from the right foreleg.

aVL - the electrode records the potential from the left foreleg.

aVF - the electrode records the potential from the left leg.

C. Unipolar Precordial Leads

CR_6L - the exploring electrode is connected at the 6th lower left intercostal space at the bottom (near the edge of the sternum) at the apex of the left ventricle. The indifferent one on the right foreleg.

CR_6U - the exploring electrode is located at the 6th left intercostal space at the costochondral junction over the free wall of the left ventricles. The indifferent one on the right foreleg.

CR_5 - the exploring electrode is at the 5th right intercostal space near the edge of the sternum over the free wall of the right ventricle. The indifferent one on the right foreleg.

CR_6L, CR_6U, and CR_5 as established by Lanneck against Wilson's central terminal (V).

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THE EFFECT OF FISSION PRODUCTS AND AGING ON THE ELECTROENCEPHALOGRAMS OF THE BEAGLE

by
H. C. Redman and J. E. Weir

INTRODUCTION

Encephalograms are being recorded on beagles prior to exposure to fission products by inhalation and at intervals post exposure. They are obtained in conjunction with a general physical examination to aid in assessing the effects of ionizing radiation should these in some measure involve the function of the nervous system. It is necessary to establish a baseline so that any effects of age can be coevaluated with those of radiation when and if they are encountered. Relevant information in the literature is limited.[1-4] Most data not only have been derived from mixed breeds of dogs of varied sex and age, but have been collected under a variety of experimental conditions. This paper is an interim report on the electroencephalographic procedures being carried out locally.

METHOD

Since frightened animals cannot be properly tested, the dogs were handled gently and placed in a canvas sling with their heads resting on a foam rubber pad and with their legs extended through holes in the sling. Only occasionally was it necessary to tie the legs of restless animals loosely to prevent undue motion during recording. It was found helpful to routinely cover the eyes using a loose gauze and cotton bandage, but no sedative or other drugs were employed.

Six 27 gauge tungsten-platinum alloy needles were inserted into the scalp so as to comprise three bilaterally symmetrical pairs in the frontal, temporal, and occipital areas. The electrodes in the temporal areas were

equidistant from those in the frontal and occipital areas. The distance between the frontal and occipital pairs were less than that between the temporal pair. The major regions of the brain were thus covered. Figure 1 illustrates the placement of the electrodes.

The electroencephalograph was a standard four channel medical instrument.* Two tracings were made in each case. The first was right frontal to right occipital, left frontal to left occipital, right temporal to left temporal, and right occipital to left occipital. This scheme permits comparison of the two hemispheres. The transverse leads permit more precise localization of electrical activities in each hemisphere.

The animals were awake when the tests were done. This was assured by hand clapping and applying physical stimulation at intervals. A total period of between one and two hours was required for each test. The instrument was frequently calibrated so that a 5 mm deflection represented 50 mv.

RESULTS

Electroencephalograms have been obtained on 32 beagles ranging in age from 10 months to slightly over a year. Details appear in Table 1.

In analyzing the records, attention thus far has been directed to dominant rhythms (frequency and amplitude), and to wave forms (prevalence and location). Table 2 shows the mean and ranges of dominant waking rhythms. Tables 3, 4 and 5 show the frequencies and amplitudes of the frontal, temporal, and occipital bipolar leads. Figure 2 shows a typical awake bipolar record of a one year old beagle.

It is felt that tracings which differ more or less markedly from these are significantly abnormal. Examples are shown in Figures 3 and 4, recorded from different animals. Both dogs are probably epileptic, but only one (Figure 4) has thus far shown even minor seizures clinically. While being tested this animal held his head rigidly cocked to the right side.

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*Electronic Medical Instrumentation System manufactured by the Minneapolis-Honeywell Co.

BIPOLAR RECORDING PLACEMENT

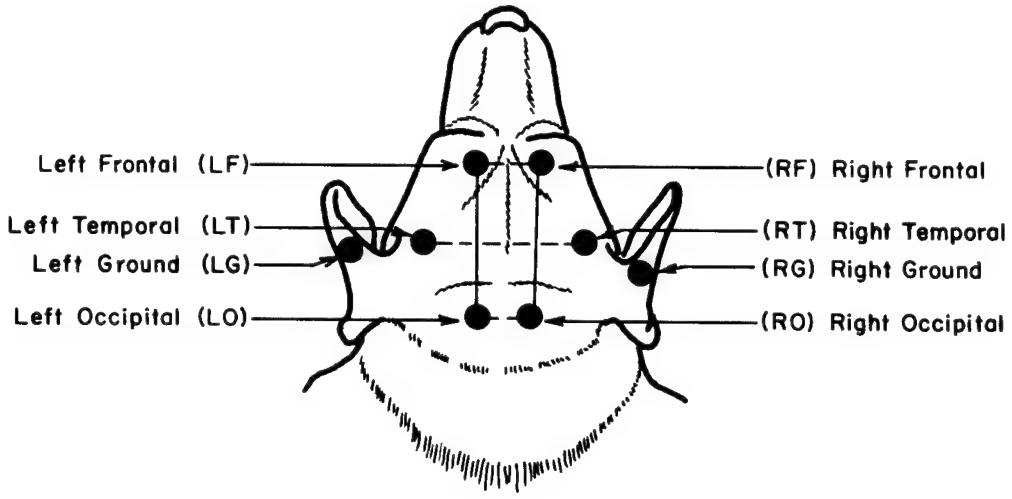

FIGURE 1

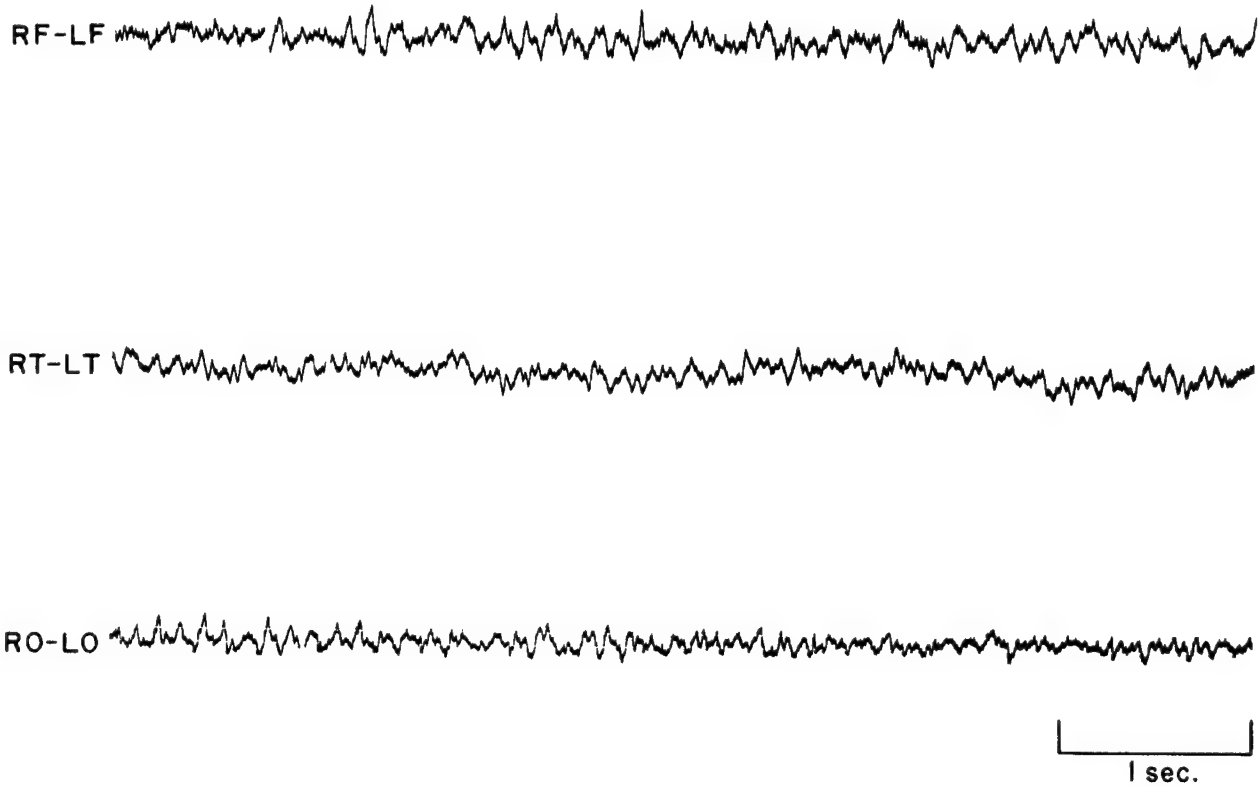

FIGURE 2

TABLE 1

THE AGE OF BEAGLES AND THE NUMBER OF ELECTROENCEPHALOGRAPHIC RECORDS OBTAINED

Age	Number of Records
10 months	1
10 months, 3 weeks	2
11 months	8
11 months, 1 week	1
11 months, 2 weeks	9
11 months, 3 weeks	2
12 months	3
12 months, 2 weeks	1
12 months, 3 weeks	5

TABLE 2

DOMINANT WAKING RHYTHM

(RF-RO, LF-LO)

AGE	No. of Records	Frequency (Cycles per Second)		Amplitude (Microvolts)	
		MEAN	RANGE	MEAN	RANGE
10 Months	1	7.5	5.0 - 10.0	16.0	5.0 - 27.0
10 Months, 3 Weeks	2	8.4	6.0 - 12.0	18.5	5.0 - 32.0
11 Months	7	8.8	6.0 - 11.5	42.6	11.8 - 53.3
11 Months, 1 Week	1	8.0	6.0 - 10.0	40.0	10.0 - 70.0
11 Months, 2 Weeks	9	7.1	4.9 - 10.1	43.0	16.0 - 70.0
11 Months, 3 Weeks	2	8.7	5.0 - 12.5	18.0	5.0 - 33.5
12 Months	3	9.0	7.3 - 11.3	33.5	10.0 - 63.0
12 Months, 2 Weeks	1	7.5	5.0 - 10.0	20.0	10.0 - 30.0
12 Months, 3 Weeks	5	8.7	5.0 - 12.4	21.0	6.0 - 36.0

TABLE 3

FRONTAL (RF-LF)

AGE	No. of Records	Frequency (Cycles per Second)		Amplitude (Microvolts)	
		MEAN	RANGE	MEAN	RANGE
10 Months	1	7.5	7.0 - 8.0	14.0	5.0 - 23.0
10 Months, 3Weeks	2	9.1	7.5 - 10.7	20.3	11.0 - 29.5
11 Months	5	8.1	5.0 - 9.2	22.3	11.0 - 43.6
11 Months, 2 Weeks	8	9.5	6.0 - 13.0	34.6	15.6 - 53.7
11 Months, 3 Weeks	2	10.3	8.0 - 12.5	15.0	5.0 - 25.0
12 Months	3	9.2	7.3 - 11.0	23.8	10.0 - 37.6
12 Months, 3 Weeks	5	7.4	5.6 - 9.2	18.7	6.0 - 31.4

TABLE 4

TEMPORAL (RT-LT)

AGE	No. of Records	Frequency (Cycles per Second)		Amplitude (Microvolts)	
		MEAN	RANGE	MEAN	RANGE
10 Months	1	6.0	5.0 - 7.0	11.5	5.0 - 18.0
10 Months, 3 Weeks	2	7.5	5.0 - 10.0	19.5	10.0 - 29.0
11 Months	5	7.5	6.0 - 9.0	33.0	13.0 - 53.0
11 Months, 2 Weeks	8	6.3	4.9 - 7.7	28.8	15.6 - 42.1
11 Months, 3 Weeks	2	5.8	5.0 - 6.5	13.8	5.0 - 22.5
12 Months	3	9.5	8.0 - 11.0	26.2	10.0 - 42.3
12 Months, 3 Weeks	5	6.6	5.0 - 8.2	19.1	6.0 - 32.2

TABLE 5

OCCIPITAL (RO-LO)

AGE	No. of Records	Frequency (Cycles per Second) MEAN	Frequency (Cycles per Second) RANGE	Amplitude (Microvolts) MEAN	Amplitude (Microvolts) RANGE
10 Months	1	8.5	7.0 - 10.0	11.5	5.0 - 18.0
10 Months, 3 Weeks	2	9.1	6.5 - 11.8	15.0	7.5 - 22.5
11 Months	5	10.3	8.2 - 13.4	64.0	14.0 - 52.0
11 Months, 2 Weeks	8	8.3	6.9 - 9.6	28.3	15.0 - 41.5
11 Months, 3 Weeks	2	10.8	9.0 - 12.5	16.8	5.0 - 28.5
12 Months	2	10.3	9.3 - 11.3	28.6	11.6 - 45.5
12 Months, 3 Weeks	3	11.0	9.6 - 12.4	19.1	6.0 - 32.2

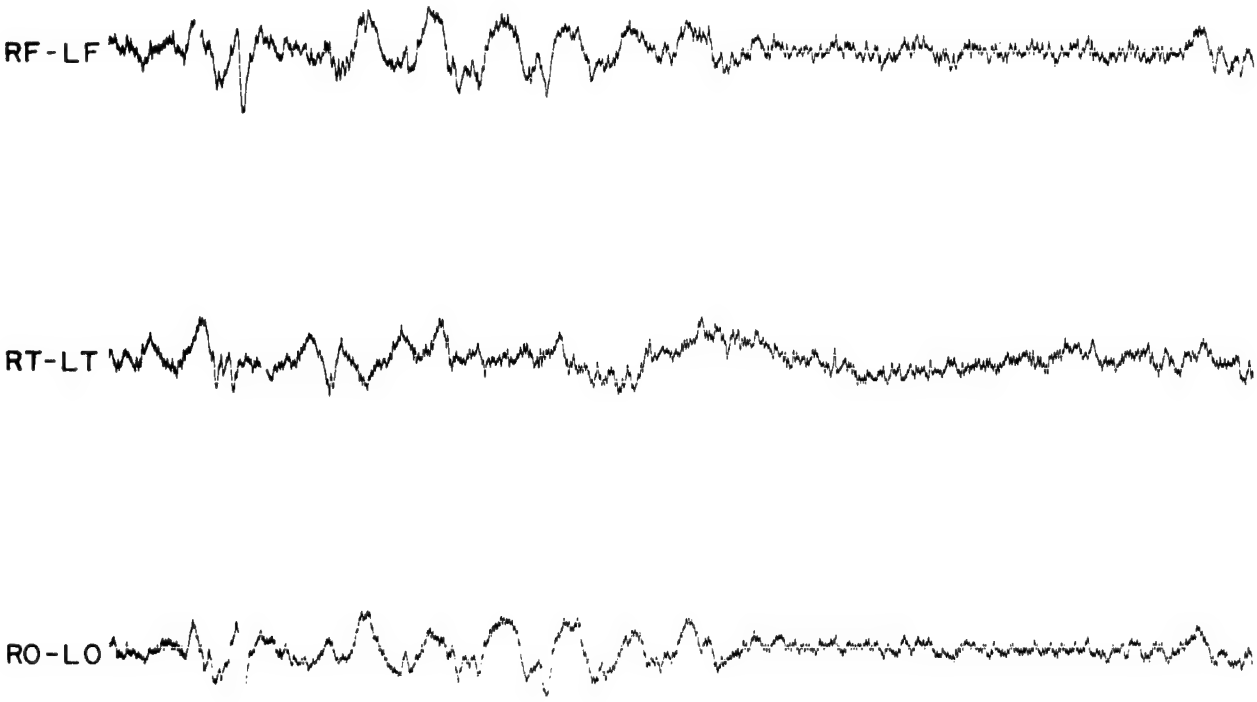

FIGURE 3

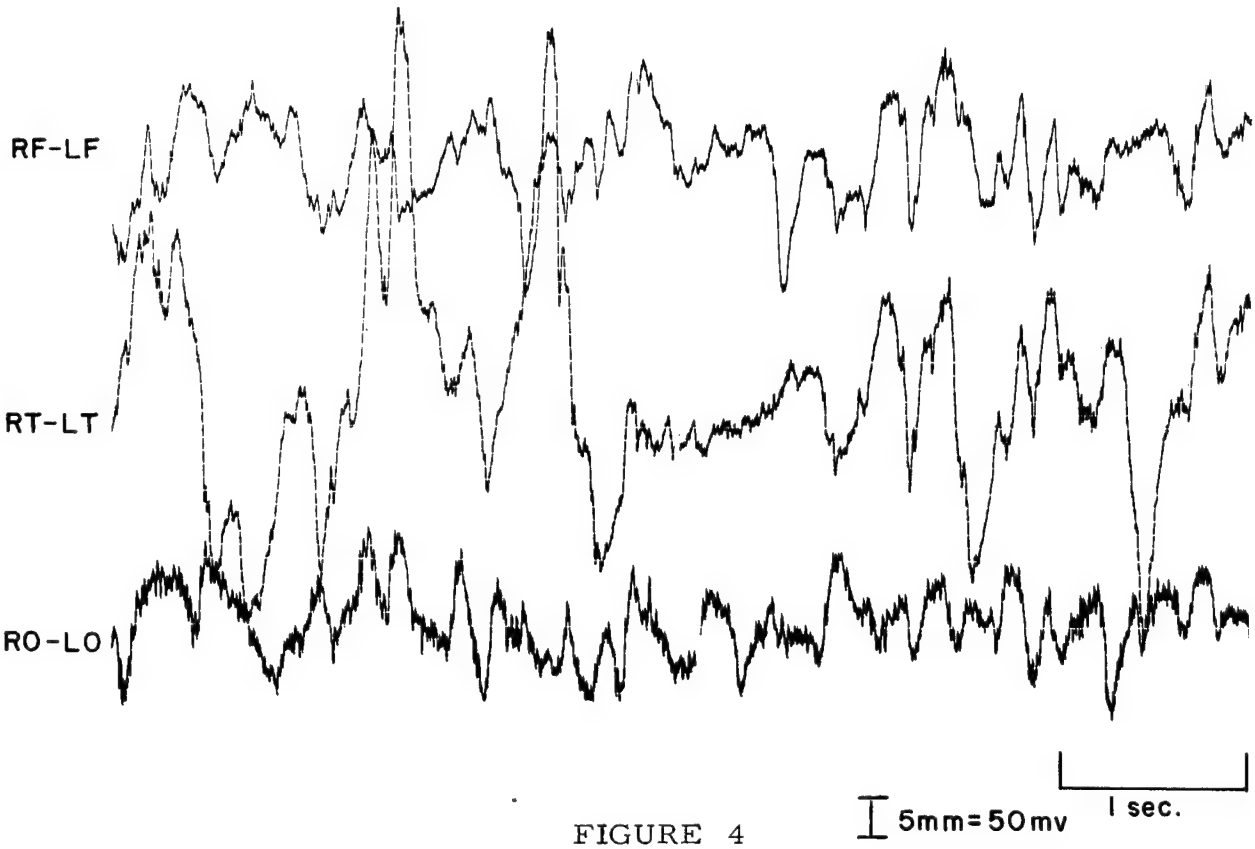

FIGURE 4

During the routine examination, three dogs from one litter showed cerebral dysrhythmia. This consisted of medium to very high voltage waves of a basic 1 - 2 cps frequency intermingled with 3 - 5 cps waves. Occasionally sinusoidal medium voltage waves at 4 - 5 cps were seen. These had durations of between 6 - 7 seconds. None of these animals harbored parasites or were manifestly diseased. They are under continued observation.

DISCUSSION

The occurrence of idiopathic epilepsy in dogs has been described,[1-2] and death from the malady has been recorded. Generalized convulsions are known manifestations of several diseases of the nervous system and of a number of systemic diseases which are not primarily neurologic. Symptomatic, as contrasted with idiopathic epilepsy, can be associated with trauma, infection parasitism, neoplasia, and toxic states. Encephalograms taken, when such maladies are present, will show various types of dysrhythmia such as spike discharges, dome wave discharges, paroxysms of slow or fast electric activity, or perhaps a generalized variation of basic rhythms. These same changes are seen in animals who have idiopathic epilepsy, except that in the latter case there is often no other manifestation of poor health, or abnormality including the occurrence of frank convulsions.

CONCLUSIONS

Though it is obvious that the electroencephalographic work will have to be continued for many years to establish age correlated changes, it is clear now that should idiopathic epilepsy appear early or late in the animals employed in the fission product inhalation studies, a serious economic and scientific loss will ensue. It is hoped that this can be avoided by detecting a predisposition to this disease early in life through routine electroencephalographic examinations.

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LONG TERM EFFECTS OF INTRAVENOUSLY INJECTED I^{131} ON RAT THYROID AND PITUITARY GLANDS

T. L. Chiffelle and J. K. Scott

Previous studies with rats show negative findings in the thyroid and pituitary glands when single intravenous doses of I^{131} ranging from 18 to 30 μc were injected into rats.[1,2] Earlier experiments in this laboratory with low doses (15-25 μc) of inhaled radioiodine have shown definite though transient histopathologic changes in these two glands. They were first observed two months following the exposure of the animals.

Thyroid effects consisted principally of a reduction in the average follicle size, and of hypertrophy, rather than hyperplasia of the follicle epithelium. Concomitantly, the colloid content was reduced, and showed a characteristic failure to stain with acidophilic dyes. The increase in cellular dimensions, which was seen, involved both cytoplasm and nucleus; but there was no overall increase in the size of the thyroid gland.

The changes in the follicle epithelium were apparently temporary, since the gland had essentially reverted to a normal appearance at the end of four months. However, at this time, the pituitary, which had heretofore appeared to be normal, showed small central collections of hypertrophied, swollen beta cells in the central portion of the gland. Such elements have previously been ascribed to the production of thyrotropic hormone; and their appearance is well known to be associated with thyroid hormone deficiencies of varying degrees.

None of these observed effects can be attributed to dietary causes; particularly since they are transient.

A second series of rats exposed to five times the dose administered to the first group showed similar changes. However, they were noted earlier and persisted longer. Whether the thyroid and pituitary cytologic architecture will eventually return to normal in this group is not known as of this writing, since the experiment is still in progress.

Because of variations in absorbed dose, when an isotope is administered by inhalation, animals may assimilate considerably more, or less than an intended amount. Therefore an ancillary experiment was initiated in which three groups of rats were given three intravenous doses of I^{131}, in order to more precisely control the amount of isotope entering the body. The animals were given 15, 25, and 45 μc respectively. They are being periodically sacrificed and observed for one year post-exposure.

The initial measured body burdens for the three groups of animals is shown in Table I. At the 15 μc level there was a difference of 14.29 μc between the minimum burden of 15.1 μc and the maximum burden of 29.39 μc. At the 25 μc level there was a difference of 6.2 μc between the minimum and the maximum. Finally, at the 45 μc level a difference of 16.5 μc existed. As expected, however, there was less overall variation in body burdens than would have resulted had the isotope been given by inhalation.

At the projected 15 μc level, 12 of 14 animals had an initial body burden between 15 and 20 μc, a variation of only 5 μc. The two exceptions had burdens between 29 and 30 μc.

At the 25 μc dose level, the variation between minimum and maximum was much narrower. In this group, 12 of 13 animals have shown body burdens between 18 and 34 μc; which is a difference of only 6 μc. Only one animal had absorbed an amount ranging between 34 and 35 μc.

In the 45 μc dose group 11 of 14 animals have shown a variation between 49 and 55 μc, a difference of 9 μc. The other 5 animals have ranged between 39 and 49 μc.

Histologic evaluation of the tissues from these animals is incomplete, as of the time of this writing; and studies are continuing.

TABLE I

Initial Body Burden, I^{131}, μc/animal

(Intravenous injection of isotope)

Planned Body Burden	Animal Number	Initial Measured Body Burden Test Animal A	Initial Measured Body Burden Test Animal B
15 μc	22	17.2329	15.8564
	25	18.4631	15.2595
	28	19.9720	15.1344
	31	18.0943	17.5806
	34	18.1273	17.5987
	37	29.3892	29.0503
	42	17.4734	19.9423

Initial Body Burden, I^{131}, μc/animal

(Intravenous injection of isotope)

Planned Body Burden	Animal Number	Initial Measured Body Burden Test Animal A	Initial Measured Body Burden Test Animal B
25 μc	23	29.1213	
	26	32.8028	28.0673
	29	30.6738	31.6207
	32	31.6728	32.2352
	35	32.8653	31.7285
	38	33.3148	32.7467
	43	34.1677	33.9905

Initial Body Burden, I^{131}, μc/animal

(Intravenous injection of isotope)

Planned Body Burden	Animal Number	Initial Measured Body Burden Test Animal A	Initial Measured Body Burden Test Animal B
45 μc	24	39.6886	53.9388
	27	43.8146	50.7153
	30	50.6754	45.5560
	33	50.3970	46.9885
	36	52.2127	49.9710
	39	53.8274	47.5043
	44	54.2460	51.4967

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LONG TERM EFFECTS OF INHALED RADIOIODINE AND RADIOSTRONTIUM IN RATS

T. L. Chiffelle and J. K. Scott

INTRODUCTION

The following report has been written to detail and summarize experimental work conducted by the Department of Pathology for the year ending June 30, 1965. In the first year of operation, this laboratory was concerned with construction, acquisition and training of personnel and with the refining of technical procedures utilizing a pilot study of Cs-137 effects on rats injected intravenously. Although portions of this section of this report will contain material presented in 1964, some repetition is necessary but is being minimized. Most of the experimental studies reported here were performed in collaboration with the Departments of Radiobiology, Biochemistry and Hematology. Results of their studies are distributed through this report.

Purpose and Objectives

Biologic effects of internal emitters generally differ from those produced by uniform external radiation because of concentration at target sites where high local dose rates prevail. Other important factors in long term effects are the turnover rate and biochemical properties of the isotope.[18, 20] The ultimate purpose of this Program is to assess long range effects of inhalation exposure to fission products, singly and in various combinations, at dosages generally less than required for acute effects. Therefore, effects quantitatively evaluated are more apt to be reduced life expectancy and neoplasia. The pathologist is, therefore, concerned with:

1. Patterns of growth and development;
2. Processes leading to atrophy and fibrosis;
3. Alterations in nutrition and metabolism;
4. Response to injury and subsequent repair;
5. Resistance to effects of micro organisms;
6. Effects on immune mechanisms;
7. Development of neoplasia.

Such evaluation entails an integration of information regarding:

1. Isotope properties, manner of absorption, tissue distribution and local concentration, metabolic turnover and excretion.
2. Direct tissue effects at local target sites.
3. Indirect effects or sequelae induced in other tissues or organs resulting from radiation and/or elemental chemical activity.

Comparing effects of a combination of isotopes is compounded by using the inhalation route and hence, the manner in which each isotope is handled by the lung. Initial experiments with "high" and "low" doses were designed to assess single isotope distribution and excretion patterns, anatomical and biochemical changes and life-span. High-dose levels were expected to affect growth rate, reduce life-span, lower resistance to infection, produce or accelerate development of tumors and induce hematologic abnormalities. Lower doses were expected to involve premature aging, atrophies and to a lesser extent, neoplastic changes and hematologic aberrations. In some instances the inhalation route imposes limitations on the magnitude of high-level exposures due to isotope costs, increased personnel hazards in the exposure procedure and subsequent care of animals.

Soluble compounds of Cs-137, Sr-90 and I-131 were selected for the early studies because of their ease of handling. Cs-137 and Sr-90 represent a particular challenge because of the long physical half-life (>25 years).[8, 9, 18, 20] Their types of radiation and associated energies are expressed below:

Cs-137	:	β-0.52 mev γ-0.66 mev
Sr-90	:	β-0.54 mev
I-131	:	β-0.6 mev γ-0.36 mev

Cs-137 is widely distributed and is expected to produce effects similar to total body X-irradiation. Chemically it behaves like potassium, having broad intracellular distribution. Approximately 60% of the absorbed dose appears in muscle with additional concentration in brain, visceral organs, blood, skeleton and teeth.

Sr-90 is rapidly absorbed and physiologically behaves as calcium. It is deposited erratically in the skeleton and particularly concentrates at sites of active bone growth. Therefore, the uptake and initial retention is much greater in younger animals, and the excretion pattern varies considerably with age. Most of the material is found in the urine. Generally, within 20-30 days after injection, there is a rapid excretion of approximately 70% of the isotope. Thereafter, excretion rate is reduced and 10-15% of the isotope is present for prolonged periods. Local skeletal accumulation of Sr-90 has profound effects on growth and on the hematopoietic system and is an important stimulus to the development of fibrous dysplasia, bone tumors and leukemia.[8, 9, 20]

I-131 is a highly effective fission product. Its primary formation at fission is small and most of it accumulates from the rapid decay of precursors.[5] It is highly soluble, has a short physical and biological half-life and between 7 and 12 % is uniquely concentrated in the thyroid gland.[6, 7] This results in a high local rad dose in spite of the short biologic half-life. Damage to the gland with suppression of hormone output may have wide spread effects on cellular metabolism and energy transfer throughout the animal.[2, 3, 13, 14, 18, 24]

Methods

Standard histopathologic methods were applied to a variety of tissues from each animal, as well as autoradiography and selected histochemical methods for detection of enzymes (acid and alkaline phosphatase, succinic dehydrogenase (SDG) and DPNG-diaphorase). Rationale for tissue enzyme studies and for a selected system has been explained.[24] With bone-seeking isotopes methacrylate embedding for sectioning undecalcified bone was

used to offset isotope loss. Cytogenetic analyses were conducted on colchicine treated rats.

Experimental Design

The study was divided into two parts: (1) a search for pathologic changes in an exposed group of rats which were serially sacrificed and (2) observations on a similarly exposed group which was allowed to live out their life span to obtain survival data. Each group was compared to matching control animals. These groups consisted of approximately 120 test animals and 120 controls. The number of animals in a group depended upon the number sacrificed at a time, the number of sacrifices and the expected maximum survival time at that dose level. With the larger animals (Beagle dogs), the number in each exposure group was governed by the available colony population coming of age (13 months).

Isotope dosage was planned for levels which would shorten life span by varying lengths, but not generally encroach upon a range leading to an acute radiation syndrome. In some instances the upper limit was governed by the capabilities of the aerosol generating system.

Longevity study groups were generally composed of approximately half the number of animals used in serial sacrifice studies. At this time only a few have died; whenever possible the deceased were dissected for histopathology. At sacrifice the test animals and controls were anesthetized with Nembutal and exsanguinated; chemical analyses on serum were performed by the Biochemistry Department and work on blood and bone marrow was performed by the Section of Hematology. Dissections were made on each animal and tissue sections were fixed in either 10% buffered formalin or formol-sublimate. Tissues selected for enzyme studies were quenched in isopentane at liquid nitrogen temperatures and so stored until processed. An example of the tissues examined is presented in Table 1. Where indicated, as in Sr-90 exposures, the entire carcass was X-rayed and the films surveyed for tumors, fractures and other skeletal alterations. Cytogenetic studies were conducted on animals injected with an appropriate dose of colchicine 1 - 2 hours before sacrifice.

Table 1

Histopathology Tissue List

Brain	Salivary gland	Kidney	Thyroid
Heart	Esophagus	Ur. bladder	Trachea
Lung	Stomach	Testes	Larynx
Thymus	Duodenum	Epididymis	Parathyroid
Lymph Nodes	Ileum-3 sites	Prostate	Adrenal
Cervical	Colon-2 sites	Seminal vs.	Pituitary
Mesenteric	Pancreas	Skeletal MM	Nerve ganglia
Spleen	Liver	Tongue	Bone-femur
Bone Marrow	Mesenteric	Diaphragm	-vertebra
	Fat	Psoas	Spinal cord
	Blood vessels	Periph. nerve	Skin

[Fixatives: Buffered formalin-10%; 10% formol-sublimate]

Tissue List For Enzyme Studies [cryostat techniques]

Thyroid, including parathyroid, esophagus, soft tissues of neck
Salivary gland
Stomach, distal portion
Duodenum and pancreas
Ileum
Liver
Spleen
Kidney

Autoradiography Tissue List

Thyroid, etc.	Spleen
Lungs	Lymph node
Liver	Blood
Kidney	

(Performed on fresh frozen tissue sections for the first 2 month sacrifices only; emulsion exposures varied from 3 days to 3 weeks.)

IODINE INHALATION EXPERIMENT

Experimental

1. Studies following Tracer Level Exposure - Serial Sacrifice

One-hundred male Holtzman-strain rats weighing 150 grams (age 50-60 days) were exposed by inhalation to an aerosol containing I-131 for a planned absorbed dose of 0.08 μc/gm. body weight. Five test and 5 control animals were sacrificed at intervals of 24 and 48 hours, 4, 8 and 15 days and 1, 2, 4, 8, 16 and 32 months. All were examined grossly for pathologic lesions and 2 experimental and 1 control were examined for histopathology. Chromosomal analyses were performed on the marrow from 2 companion test animals and 1 control. These animals were subcutaneously injected with 1 ml. aqueous colchicine solution (0.75 mgm/ml) one hour prior to sacrifice and fresh femoral marrow was collected into Difco 199 culture medium. Tissue samples for enzyme studies were treated as described.

2. Serial Sacrifice following Higher Dose Exposure Studies.

One-hundred and twenty similar Holtzman-strain rats were exposed to I-131 for a planned absorbed dose of 0.4 μc/gm. body weight. Procedures for sacrifice and examination were identical to the above.

3. Longevity Studies

Two groups of rats, one at each dose level of I-131 (0.08 and 0.4 μc/gm) were exposed by inhalation simultaneously with the serial sacrifice groups. The disposition of animals is enumerated below.

Isotope Dose/gm	Number of Animals: Males	Females	Total
0.08 μc	9	50	59
(controls	9	50	59)
0.4 μc	-	49	49
(control)	-	49	49)

Animals in this study were permitted to live to accumulate statistical survival data and correlative information concerning cause of death in exposed and control animals. To date, very few rats in this series have succumbed. When possible all dead animals were thoroughly examined and resulting information was tabulated. Special interest was attached to the matching control group because statistical information concerning naturally occurring diseases in the Holtzman-strain has not been well documented.

4. Tracer Dose Range Effects in the Thyroid – Pituitary

In the initial pathology evaluation of serially sacrificed animals up to 160 days after inhalation exposure to tracer levels of I-131 (0.08 μc/gm) there appeared to be minimal but distinct cytologic changes noticeable in the thyroid at 60 days and in the pituitary at 132 days. This unexpected finding was of particular interest because it occurred long after disappearance, as determined by autoradiography, of iodine from the thyroid gland. Previous observers (Durbin and Goldberg, et al.[6, 10]) have not described tissue effects at levels from 0.07 to 0.33 μc/gm. body weight.

It seemed possible that the animals had absorbed an I-131 dose considerably greater than the intended level and that minimal histologic changes had resulted. Therefore, an ancillary experiment was designed to bracket these doses and, to avoid the usual variation in inhalation dose, the isotope was administered intravenously. Forty-eight rats like those used previously were divided into 3 groups according to the following regime:

μc/gm	μc/animal	Number of test animals	Number of controls
0.09	15	16	8
0.165	25	16	8
0.297	45	16	8

Two test animals and one control in each of the three groups were sacrificed at 2, 4, 8, 16, 32, 64, 128 days and 1 year after injection. They were examined grossly and histologic examination of thyroid and pituitary glands was performed. Tissue enzyme studies were conducted on thyroid

gland and adjacent structures, in a single block, following preparation by cryostat technique. Paraffin embedded tissues were stained with hematoxylin-eosin. Pituitary gland was stained by the periodic-acid-Schiff, PTAH, and a trichrome method for differentiating the various cell types. Autoradiographs were made of cryostat sectioned thyroid tissue up to day 64.

Results

1. Control Animals

Histologic examination of tissue from control rats showed no distinctive deviation from expected findings. Particular attention was directed to alterations in the respiratory tree, rate and character of bone growth, status of lymphoid and hematopoietic tissue, changes in thyroid, pituitary and other endocrine organs and relative frequency of mitosis in various tissue structures.

Focal respiratory tract inflammations are not uncommon in rodents and appear to increase with age. In these animals the incidence of respiratory changes was minimal and no increase was seen. Most frequent findings were scattered lymphoid nodules in lungs, adjacent to bronchial structures, and varied degrees of chronic bronchitis and bronchiolitis; mucosal infiltrates of lymphocytes and plasma cells in the larynx and trachea were present. One animal currently has developed a marked bronchiectatic pulmonary inflammation with areas of pneumonia and abscess formation at approximately 100 days of age. Chronic tracheitis and laryngitis was common in many animals and the degree of inflammatory change varied widely showing no significant difference between test and control animals.

Appearance of lymphoid tissue including thymus, lymph nodes and spleen was not remarkable. Generally, as the age of the animal increased, cervical and thoracic lymph nodes were slightly larger, probably reflecting hyperplastic response to microbiologic activity in the upper and lower respiratory tract.

Bone growth appeared orderly. In the young animals (age 50-60 days) cartilaginous epiphysis and metaphyseal spongiosa was broad with active osteoid formation. Aging, with decreasing growth rate became evident at age 100-180 days with narrowing of epiphysis, depletion of subepiphyseal spicules and trabeculae and decreased osteoblastic activity. At 200-240 days most epiphyseal activity had ceased. Bone marrow changes (femoral and vertebral) were limited to normal aging depletion in which marrow became increasingly fatty beginning at the 100th day of age. Fat replacement of marrow at age 300-325 days was usually quite marked. Hematopoietic elements in tissue sections of bone appeared to exhibit a population distribution within normal limits, irrespective of the degree of fat replacement.

Microscopic structure of control thyroid glands was quite constant during the study. Average follicle size varied from 50-180 μ diameter. Height of the lining epithelium generally varied from 6.5-9.0 μ for smaller follicles and 4.5-6.0 μ for the larger. Most were generously filled with weakly acidophilic colloid but inflammatory cells were not present.

Cytologic characteristics of the pituitary gland were not remarkable in any control animals at 348 days after start of the experiment (approximate age of 400 days). Alpha and intermediate type cells, as well as chromophobic cells, dominated the cellular distribution. Beta cells were extremely scarce, consistent with observations of Purves and Greisbach and of Goldberg and Chaikoff.[11,19]

Extent of mitotic activity was assessed in various tissue structures mainly to compare test animals at different time intervals. Mitotic figures are well known for actively regenerating tissue such as intestinal mucosa, lymph nodes, spleen, thymus, bone marrow, testes, skin, and respiratory epithelium. They are also common in renal tubular epithelium, thyroid gland and pituitary. Proportionate numbers seen in control animals appeared relatively constant to the age of 400 days.

2. Autoradiographs

Of the autoradiographs performed on several tissues only those of the thyroid gland were of interest. Results at both dose levels of the isotope

were essentially constant. To day 4 after exposure the thyroid mass was a solid blackened area presumably due to the isotope. At day 8, as seen in Fig. 1, the total isotope concentration had considerably decreased but heavy concentration sites remained scattered throughout, particularly in larger follicles. By day 16, most of the isotope had disappeared (Fig. 2) leaving only rare isolated "hot spots." All I-131 had disappeared from the thyroid by 32 days after exposure.

3. Initial Body Burden

The known variations in isotope absorption administered by inhalation, is illustrated in Table 2 by the measured initial body burden of I-131 in the sacrificed animals of both the "low" and "high" dose series. In Table 2 (low dose) these ranged from 4.32 to 22.22 μc, representing a variation of 5.3 compared to an expected burden* of 12-12 μc/animal.

The higher dose was expected to result in a body burden ranging from 60-69 μc for these 150 gm rats.

Measured values ranged from 5.45 to 125.22 μc, as shown in Table 3. Only one test animal had a recorded value of 5.45 and the next lowest contained 36.96 μc. Except for the one low value, the dose varied by 5.1. The number of animals tabulated here would not bear statistical criticism, however, it is of tentative interest that in the "low" dose group 37% deposited less than expected, 26% were within the expected range and 37% deposited greater than expected. At the "higher" dose 23% of the rats absorbed less than expected, 15% were within the projected range and 62% were above.

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*Expected burden was arbitrarily derived from related exposures when a similar dose was delivered intravenously. Eighty-five per cent of these animals showed a body burden varying by only 5 μc.

Table 2

Initial Body Burden, I^{131}, μc/animal

[Calculated inhalation exposure dose: 0.08 μc/gm]

Animal Group Number	Test A	Test B	Sac. Days After Exposure
3	4.3210	9.0608	24 hrs.
4	9.2509		48 hrs.
5	13.7491		4 days
6	20.4100		8 days
7	18.2006		16 days
8	15.3409	11.2208	32 days
9	15.8633	6.2840	62 days
17	15.6202	16.7411	131 days
18	11.8671	5.4865	160 days
40	15.8182	18.5596	330 days
61	22.2193		467 days
62	18.8647		469 days
69	16.7097		502 days

Table 3

Initial Body Burden, I^{131}, μc/animal

[Calculated inhalation exposure dose: 0.4 μc/gm]

Animal Group Number	Test A	Test B	Sac. Days After Exposure
11	108.5062	69.8134	24 hrs.
12	94.6242	62.6707	48 hrs.
10	82.5434	64.1913	4 days
13	79.2144	93.1952	8 days
14	125.8337	123.9045	16 days
15	56.0242	124.6862	32 days
16	5.4552	126.2194	64 days
19	108.2868		94 days
20	105.9694	46.5824	131 days
21	36.9558	85.4021	188 days
41	61.0791	84.8539	276 days
49	91.9973		348 days
50	56.2278	68.3835	362 days
53	79.0903		367 days
54	40.3084		377 days

4. Histologic Findings

a. Low Dose (0.08 μc/gm)

Microscopic changes were minimal and were confined to the thyroid and pituitary gland. At 16 days post exposure no visible cytologic effects were seen although no mitoses were found in the thyroid epithelium. In the 16 and 32 day period, thyroid follicle epithelium appeared slightly taller with some cytoplasmic vacuolization. A few pyknotic nuclei were found but not in greater frequency than in the controls. Mitotic inhibition persisted in thyroid epithelium. No inflammatory reaction was present, nor were there any tissue alterations observed in related structures (blood vessels, connective tissue, parathyroid gland, etc.). Except for the mitotic inhibition, described alterations were probably of questionable significance with respect to the rather well known "normal" variation in thyroid structure.

The first well defined microscopic thyroid changes were seen at day 62. Follicle diameters were reduced, colloid content decreased and was often quite scanty and epithelial cells were increased in height. There was occasional nuclear pyknosis and cellular desquamation, but the significance of this was doubtful since similar changes were sometimes seen in control material. The pituitary gland was not remarkably changed.

In subsequent sacrifice periods at 131, 160 and 330 days thyroidal alterations were inconsistent and minor. It was difficult to define significant differences between control and test material. At 131 days the pituitary of experimental animals showed slight increase in number of large beta cells but this trace difference disappeared at 160 and 330 days. No histologic changes were seen in other tissues that could be directly related to the isotope, even in the early stages of the experiment when high concentrations existed in the circulating serum and kidney, the main route of excretion. Tissues with high cellular turnover showed no reduction in mitotic frequency nor detectable aberrations in the mitotic apparatus. Even the pituitary gland, where some isotope accumulates, failed to show evidence of direct radiation effect. No cytologic alterations were ever seen

in the renal tubular epithelium, nor was there reduction in the expected number of mitoses. Although the respiratory system was the primary site of deposition of the I-131, the rapid absorption apparently precluded detection of any cellular changes. Aging in test animals and controls appeared comparable, including maturation, changes of femoral epiphyseal plate, progressive fat replacement of marrow and focal renal sclerosing (glomerular mesangial thickening, tubular atrophy and slight thickening of afferent arterioles). Bone and marrow changes were first seen in the 62-160 post-exposure period, after renal changes were noted at 330 days. Peripheral blood smears and sections of marrow showed no distinctive changes.

3. High Dose (0.4 μc/gm)

In this group the initial body burden of I-131 was greater than expected for the majority and in some instances reached 125 μc. Again, microscopic changes were principally seen in the thyroid and pituitary gland. Traces of thyroid follicle change were apparent at 16 days in contrast to 32 days at the "low" dose level. Follicles were smaller, colloid scantier and epithelial cells taller than in controls. Early sacrifices showed only mitotic inhibition but at 16 days one abnormal mitotic figure was found; notably these test animals had nearly double the expected body burden. There were no associated inflammatory reactions and adjacent tracheal epithelium showed no mitotic inhibition. Pituitary glands of test and control animals were alike and unremarkable.

At 32 days thyroid changes were more marked than previously even though the isotope had left the gland. Follicle diameters ranged from 50-94 μ and the epithelial lining increased in height (14-18μ). Nuclei were enlarged and more variably sized. There was some nuclear pyknosis and desquamation of epithelial cells. Colloid was scant and appeared to stain abnormally. In the adjacent tracheal lymphoid tissue, nuclear fragmentation was found. In the pituitary, rare large vacuolated beta cells were seen but these were numerically judged to be of questionable significance.

Thyroidal changes continued to the 188th day of sacrifice and observations are continuing. Early changes are illustrated by comparing a sample control (Fig. 3) and test animal (Fig. 4). The anterior hypophysis exhibited significant accumulations of large, swollen beta cells (thyrotropic) in the more central portion of the lobe, as described by Purves and Greisbach and by Goldberg and Chaikoff.[11,19] The presence of these cells was in marked contrast to the control glands as illustrated in Figs. 5 and 6, but their presence has somewhat diminished by 188 days. Concomitantly, alpha cells were numerically reduced and were slightly degranulated, although this change was not particularly striking. Some differences in magnitude of thyroid and pituitary changes in test animals could usually be related to differences in deposited isotope.

No significant changes were seen in other tissue except for progressive aging changes in bone, marrow and kidney, as described previously for the "low" dose series. A differential bone growth rate was observed at 131 and 188 days. Exposed animals showed reduction in epiphyseal plate and reduction in metaphyseal spongiosa in both femur and vertebrae. One control animal had marked chronic respiratory inflammation with multiple micro abscesses (at 64 days). With age occasional lymphoid aggregations appeared in the prostate gland. One test animal (No. 19) was prematurely sacrificed at 94 days because it appeared to be malnourished. Tissue studies of this animal showed only lymph nodal proliferation of reticular cells and plasma cells of undetermined etiology. Thyroid and pituitary gland reflected changes as described previously but other tissues were not remarkable.

5. Histochemical Enzyme Changes

Significant increases in acid-phosphatase and DPNH-diaphorase occurred in the thyroid epithelium of "low" dose animals sacrificed at 131 days but reversed to normal at 160 days. In this series, testing for tissue acid phosphatase localization was not done prior to this time and effects may have unnoticeably developed.

In the "high" dose series, similar and more pronounced increases of these enzymes in the thyroid were first noticed on the 32nd day and have persisted to the 188th day. In controls both enzymes were uniformly distributed in cytoplasm as fine particulates. In experimentals at times corresponding to the previously described follicle alterations, both enzymes were notably increased in concentration but the visible amount varied considerably between cells. These enzyme increases were particularly conspicuous (especially with the strong red colorimetric hue of the acid-phosphatase method) and seemed partially related to the increase and variation in cell size. No other tissue enzyme changes of significance were seen in liver, kidney, salivary gland, spleen, stomach, or intestine, except for decreases in the few microscopic areas of renal tubular atrophy reflecting the aging changes described previously.

Discussion

Normally some exogenous iodine (approximately 12% in 48 hours) is rapidly localized in the thyroid gland, by a series of conversion reactions with tyrosine is converted to thyroxine and tri-iodo-thyronine and is bound within the thyroglobulin of stored colloid in the thyroid follicles.[13] After injection iodine appears within 2 minutes at the luminal margin of the thyroid epithelial cell.[17] The bulk of circulating iodine is rapidly excreted in 48-72 hours.

Beside the special affinity of iodine for the thyroid, other tissues rapidly take up significant quantities of the ion. Among these are red cells, salivary glands, mammary and sweat glands and stomach, where large amounts are secreted by mucous neck cells.[3,13] Durbin[6,7] using tracer doses of I-131 in rats, has described significant concentrations of isotope in lung, kidney, pituitary, liver, gonads, spleen, adrenal, pancreas, prostrate, salivary and mammary glands. With a larger dose (50 μc/gm) highest isotope concentrations after 26 hours appeared in pelt, blood, ovary, lymph nodes, heart, lungs, kidney, liver, adrenal and spleen, in addition to thyroid concentration.

Because much iodide ion is incorporated into thyroid hormone it is expected that the amount of circulating iodine would be regulated, in part, by hypophyseal thyrotrophic hormone (TSH). A "feed-back" mechanism exists by which circulating thyroid hormone controls the secretion of TSH (via hypothalamus) which, in turn, regulates the synthesis and release of iodinated thyroid hormone.[13] Thyroid hormone action is exceptionally broad with fundamental effects at the cellular level energy metabolism affecting amino acid synthesis and transport in addition to energy availability from carbohydrates and fat. The mechanics of some of these important effects are still obscure. The complex interactions of thyroid hormones and effects of dysfunctions of the gland are described in several excellent reviews.[2,3,13,14,24] I-131 may be handled biologically different from the non-radioisotopic form because of its direct affect upon cellular metabolic processes but the long term effects usually fall into 3 general categories:[7]

(1) Damage in the vicinity of the thyroid gland.
(2) Secondary or indirect alterations due to thyroid deficiency.
(3) Direct radiation damage to tissues remote from the thyroid gland.

In the realm of single high dose effects of I-131 in rats, the work of Durbin et al., is of particular interest.[6,7] Comparisons were made between effects of tracer and higher level doses ranging from 10 to 90 μc/gm with respect to excretion, tissue concentration, growth rate, life span and pathology. Excretion pattern differences existed; in the first 4 hours after intravenous injection approximately 40% of both doses were eliminated. In the next 4-24 hour period elimination of the high dose was retarded, but thereafter, a rapid rate of elimination occurred and 96% of the dose was excreted by the sixth day. Eighty-eight percent of the low dose level was excreted by this time. There were also differences in thyroid retention of the isotope between high and low doses. Twenty-four hours after tracer doses the thyroid retained 7-12% of that injected, 5-10% at 48 hours

and thereafter it gradually fell to 2-4% by 11 days. With a high dose (50 μc/gm) thyroid retention was much the same for the first 24 hours, but was rapidly depleted to 1.5-2.5% at 48 hours and almost completely eliminated by 10 days. This rapid elimination was due to destructive effects on the thyroid gland and resulting incapacity to store the ion. Isotope doses of 10 μc/gm and above markedly reduced growth rate and life-spans dropped precipitously, particularly above 30 μc/gm, which is regarded as a "toxic threshold."

Pathologic changes were marked in all dose ranges except the tracer level. At 10 μc/gm the thyroid gland was obliterated and there was some decreased fat, lymphoid and splenic tissue. The pituitary gland showed characteristic post-thyroidectomy changes with degranulation of alpha-cells and increased numbers of beta-cells. In the range of 30-70 μc/gm there was increasing damage to parathyroid tissues, progressive depletion of splenic and lymphoid tissue, lymphopenia, granulocytopenia, marrow hypoplasia, ovarian atrophy and fibrosis, partial adrenal cortical atrophy and cytoplasmic degeneration in renal tubules.

The studies of Goldberg et al.,[10] are of more direct interest for comparison with effects seen in the present study. To ascertain a continuous picture of changes induced by radiation effects of I-131 to the thyroid gland, 4 groups of 250 gm male rats were given graded intravenous injections of the isotope in amounts of 18, 300, 525 and 875 μc/rat (equivalent to 0.072, 1.2, 2.1 and 3.5 μc/gm), considerably less than Durbin's lowest toxic level.[6] Animals were sacrificed at frequent intervals up to 3 days, weekly up to one month and monthly up to 8 months. With the highest dose level (3.5 μc/gm) there was massive destruction of thyroid tissue within 48 hours, followed by scarring and injury to the trachea. No regeneration had occurred after 6-8 months. Pituitary cytologic changes were delayed, but between 1-4 weeks alpha cells were degranulated and reduced in number and became hypertrophied with hyalin cytoplasmic vacuoles. These effects remained for the 8 months. At the dose level of 2.1 μc/gm thyroid

damage was severe but some regeneration had occurred at 8 months. Pituitary changes were essentially similar to the above without restoration to normal pattern. At 1.2 μc/gm, isotope effects to the thyroid were manifested in 24-72 hours but after 8 months the follicles appeared normal. Similar pituitary effects but of lesser degree to the above occurred in 2-4 weeks; the gland was restored to normal after 5-8 months. The tracer dose of 0.072 μc/gm failed to induce histologic changes in either thyroid or pituitary glands in one month.

Observations have presently been completed to 330 days for the 0.08 μc/gm dose group and to 188 days for the 0.4 μc/gm group. The delayed (2 months) transient change in thyroid follicle epithelium at the low dose level is of interest since essentially negative findings have been reported by previous investigators.[6,7,10,11] Perhaps the time of observation in their studies was limited. Temporary increase in the number of centrally located beta cells in the pituitary did not occur until sometime after the 2 month period and was first noticed at 131 days. The gland reverted to normal status by 160 and 330 days. During the period of isotope storage in the thyroid gland (up to 32 days) no microscopic changes were found in thyroid or pituitary glands. At the high dose level thyroid effects were observed to begin earlier (minimal at 16 days) and progressively became more pronounced. Pituitary changes were minimal at 32 days but pronounced at 62 days. The thyroid-pituitary cytologic alterations have continued up to the present time.

In the low dose range, the deposited I-131 differed little from the generally acceptable tracer levels (15-25 μc), in spite of the variation in initial body burden obtained by inhalation. At the higher dose level a larger percentage of animals (62%) received greater than the "expected", attaining levels of 125 μc in some cases. The maximum level was therefore less than half of the lowest dose used in the Goldberg and Chaikoff series in which more marked effects were seen in thyroid and pituitary and which began at a much earlier time (24 hours for thyroid change; 1-2 weeks for pituitary alteration).[10] It would tentatively appear from our data that the

range of absorbed dosage from approximately 60-125 μc/animal would be in a threshold area of prolonged effects since these have persisted for several months.

The possibility was considered that certain dietary peculiarities might have been influential in promoting the observed thyroid epithelial alterations, since sodium-sulfamethazine was introduced into the animal's drinking water in concentrations of 0.1 mg/cc (0.01%), equivalent to 5 mg daily consumption. A number of "anti-thyroid" agents, among which are thiouracil and sulfanilamide, are known to alter thyroid histology and promote reflected changes in the pituitary gland when given in sufficient amounts.[1,4,22] In general, these agents promote thyroid epithelial hyperplasia and decrease in hormone production, either by blocking the uptake of iodine or inhibiting enzyme systems involved in the production of thyroxine. Woodward and Berard have recently ascribed such effects to the poorly absorbed sulfaquanidine administered to rats in amounts of 2% of the diet.[22] Sulfamethazine is readily absorbed and rapidly eliminated. We are not currently aware of studies concerning this drug which would indicate that it might have some direct effect on the thyroid gland at the concentrations used (3.2% of a full, daily "therapeutic" dose). It appears unlikely that the drug influenced thyroid changes for the following reasons; (a) none of the observed changes were seen in the control animals which received the same diet; (b) in the low iodine dose group thyroid changes were transient and the gland of test animals seemed to have reverted to the normal pattern; and (c) the described effect of goitrogenic substances is to promote hyperplasia with increased numbers of mitotic figures. In our experimentals the thyroid gland was not increased in size and the cytologic effect was one of cellular hypertrophy not hyperplasia.

STRONTIUM INHALATION EXPERIMENT

Experimental

1. Rat Exposure - Serial Sacrifice

One hundred and twenty male Holtzman rats and 40 female rats were exposed. Average weight of the male rats was 180 grams (range 136-227

grams); whereas, female rats averaged 165 grams (range 96-188 grams). The aerosol was generated from a solution that contained 95 mc of Sr-90 and 34 mc of Sr-85 (a ratio of 28.6:1); the vector was 1% cesium chloride. Median dose that the animals received was 0.71 μc/gm (range 0.18-1.54 μc/gm) in the case of the males and the median dose was 0.705 (range 0.16-1.11 μc/gm) in the females. Nine of the exposed males and ten of the females expired within the first 24 hours following exposure and were, therefore, excluded from the experimental group.

Five test animals and five control animals were sacrificed at each designated period (1, 2, 4, 8, 15 days; 1, 2, 4, 8, 16, 32 months; and at end of life span). All animals were thoroughly examined and tissue samples from 2 test and 1 control animal were fixed and stained for histologic survey. The list of tissues examined was essentially similar to that described for I-131 exposures. Two additional test animals and 2 controls were injected wtih colchicine 1 hour prior to sacrifice, and marrow aspirates processed for chromosome analysis. Tissue enzyme analysis (acid and alkaline phosphatase, succinic dehydrogenase-SDH and DPNH-diaphorase) on two test and one control subject was performed on fresh slices of liver, kidney, spleen, stomach, and jejunum quenched in isopentane at liquid nitrogen temperature. Autoradiographs were initially performed on acetone fixed sections of femur and vertebra embedded in methacrylate resin. Other samples of femur and vertebra were fixed with buffered formalin (10%), decalcified, and processed in customary manner for general histologic details.

When clinical symptoms developed, examinations were expanded to include radiographic surveys of skeletal system of all animals, using both hard and soft x-rays, and many other bone and soft tissue samples were taken for histologic surveys. When indicated, urine cultures were taken for microbiologic assay.

Between 190 and 216 days after exposure, a large number of the exposed male rats became acutely ill necessitating premature sacrifice. Since the parallel groups of female rats for longevity study (see below),

exposed to the same levels of isotope, were noted to be clinically free of symptoms, the sacrifice periods were re-designed to compare effects between the two sexes and to permit closer intervals of effects study. Accordingly, sacrifice schedule was re-arranged on a monthly basis to involve 10 animals (5 exposed males and 5 exposed females) and 10 control animals (5 of each sex), at each period—to continue until the series was exhausted.

2. Rat Exposure — Longevity Study

Forty female Holtzman rats, 165 gram weight, were exposed by inhalation to an aerosol comprised of a mixture similar to that described for the serial sacrifice group. An equal number of control female rats was incorporated into the study. This group was permitted to live out their life span to be examined only at time of death. Because of the apparent effects differences noticed between the male sacrifice series and the female longevity series, this experiment was terminated by converting the entire number into the serial sacrifice study as described above, beginning with the 225th day after exposure.

3. Canine Exposure — Serial Sacrifice

Late in 1964, a limited number of beagle dogs became available for Strontium 90 inhalation effects studies. Since strontium is handled in a manner similar to the calcium ion, and its effects are more pronounced in the younger animal, the early adult age of 13 months for exposure was selected. Initially, it was planned to expose comparable groups of animals to a "high" and "low" level of isotope (100 μc/kg. and 33 μc/kg., respectively), both for continuous biologic effects study (serial sacrifice) and for longevity study.

Accordingly, 24 test animals (matched with 10 control animals) were exposed by inhalation of an aerosol containing a mixture of Sr^{85} and Sr^{90}, as described previously for rat exposures, attaining an administered dose of 125 μc/kg. These animals were to be periodically bled for biochemical and hematological studies at 1, 3, 7, 14, 21, and 28 days, and every month thereafter. The sacrifice schedule was tailored to place less emphasis on sampling in the early part of the expected life span, thus spreading

the examinations at more regular intervals in the mid and later part of life span. In view of the rather long life span of the dog, a sacrifice schedule similar to that for rats would have resulted in too widely spaced examination intervals, particularly at times when neoplasms and accelerated aging phenomena would most likely take place. Ten sacrifice periods were planned, limited by the total number of animals available at the time of exposure. Two test and 1 control animal were to be sacrificed at 4 days, 28 days, 6 months, 12 months, and every 4 months thereafter. Four test animals were retained in a reserve pool so that, in the event of early spontaneous death of scheduled sacrifice subjects, replacement would be possible.

Complete pathologic examinations were performed and tissue samples selected for histologic study in the customary procedure as outlined for rat exposures. Tissue fixation was accomplished with either 10% buffered formalin or 10% formol-sublimate. The skeletal system was studied radiographically, and bone samples selected according to number of lesions found. Where roentgenograph films were negative, several bone samples (femur, humerus, vertebra, etc.) were selected at random for radioautographs and microscopic study. Biochemical and hematological analysis was done immediately prior to sacrifice. Marrow samples were obtained directly from femoral medulla at necropsy. Selected tissue samples of liver, spleen, kidney, stomach, and jejunum were processed for tissue enzyme analysis, as described for rat exposure series.

The planned canine group exposure to the "low" dose level (33 μc/kg) was temporarily suspended in the effects or serial sacrifice series due to an inadequate supply of beagles.

4. Canine Exposure - Longevity Study

Paralleling the canine exposure for serial sacrifice, described above, groups of animals were assigned for exposure to the same "high" and "low" level isotope dose. However, due to the limited numbers of animals available, it appeared logical to place greater emphasis on initiating a braoder exposure spectrum for the longevity series than was originally planned; and to delay the serial sacrifice "low" level study until a later date.

Thirty-six beagle dogs, age 13 months, were divided into 3 groups of 12 animals each. Six of each group were males and 6 were females. Exposures were conducted during the months of July to October, 1965, by aerosol inhalation method, according to the following schedule.

	No. of Animals		Isotope
	Male	Female	Dosage (μc/kg)
Group I	6	6	125
Group II	6	6	25
Group III	6	6	5

RESULTS

1. Preliminary Results: Rat Exposures to Strontium

a. Gross Findings

At the present time, 156 animals have died or have been sacrificed (100 exposed and 56 controls). Among the experimental, 58 were males and 23 females. Of these, 15 have shown tumors of the vertebrae, 6 of which were multiple. Twenty-seven animals have shown tumors in bones other than the vertebrae. Most of these occurred in long bones (tibia, femur and humerus), however, one was seen in the jaw and another at the base of the skull. There were multiple tumors in a number of the animals.

The tumors varied in size from small microscopic areas to as big as 4 cm across. There were some cases of bone destruction. The larger tumors invaded and displaced surrounding soft tissues.

In the animals with vertebral tumors, there was compression of spinal cord with paralysis of the hind quarter. Urinary bladder dilatation and prostatitis was frequently seen presumably due to interference with autonomic supply to the bladder. In a few cases, there was a mild hydronephorsis. Cultures of the urine in a number of these animals showed a variety of organisms (proteus, streptococcus faecalis, E. coli, etc.).

Pulmonary metastases were observed which varied in character from microscopic nodules to masses that almost obliterated the lung. Many of

the animals showed chronic bronchitis and penumonia, commonly seen in rats, which in some cases was quite extensive.

b. Microscopic Findings

The earliest change observed in the bones was a depletion of the marrow in the region of the metaphysis (Figures 7 and 8). This was present in sacrificed animals on the 16th and 32 day post exposure, but was not seen thereafter until 127 days after exposure. At the latter time there was a different type of lesion in which the marrow of long bones and to a lesser extent of vertebrae was replaced with fat. The extent of this lesion varied widely in the bones of different animals and in a single bone. In many animals no such replacement of marrow with fat was observed in the bones examined (Figures 9, 10, 12 and 15).

In the long bones of some rats a transverse bar of unresorbed spongiosa was seen, in many it was absent; this indicates the linear growth of bone beyond the original epiphyseal plate (Figures 9 and 10). Many of the long bones showed areas of varying length, in which cortex of the shaft was thickened with dense cortical bone (Figure 15).

The exact point of origin of the tumors was impossible to determine except in a few early cases where small foci were observed microscopically in the region of the unresorbed spongiosa. All of the early tumors seemed to arise in the marrow cavity and in some cases there were multiple points of origin (Figure 26).

The microscopic changes observed in the tumors were quite variable, the lesions varying from areas of fairly well differentiated connective tissue or osteoid to highly malignant solid tumor. There were many lesions which seemed benign; others which contained atypical cells. These may have been "precancerous" and formed an intermediate group in which it was difficult for the pathologist to decide exactly what he was dealing with. In some cases these different forms occurred in the same bone. An example of this is shown in Figure 12 in which well differentiated osteoid is

adjacent to malignant osteogenic sarcoma. Higher power magnifications of the junctions of these lesions are shown in Figures 13 and 14. The structure of these malignant tumors is also shown in Figures 18 and 23. Many of the malignant tumors contained immature cartilage, similar to osteochondrosarcomas seen in humans.

The malignant tumors were sometimes limited to the marrow cavities; in other cases, bone destruction had occurred and large tumor masses invading and displacing soft tissues were seen. This was interesting in those tumors arising in vertebrae which compressed the cord (Figure 16) or invaded the cord (Figures 19 and 20). These tumors also formed large masses which invaded and displaced the surrounding soft tissues (Figures 17 and 19). In the cases of cord compression, the prostate showed interstitial fibrosis and inflammation, a purulent exudate and frequently hemorrhage in the lumens of the tubules (Figure 21) sometimes accompanied by a slight hydroephorosis with dilatation of renal tubules (Figure 22).

Metastases occurred most frequently in the lungs. These varied in size from small tumor emboli to large masses (Figures 23 and 24). A few small metastases were seen in the kidneys and adrenal (Figure 25).

Autoradiographs have been performed on the bones of most of these animals, but most have not as yet been processed (Figure 11).

The cases of leukemia are described elsewhere in this report.

2. Tentative Results: Canine Exposure to Strontium

Experimental observations are in progress at the present time. Only a few animals have been sacrificed and that data is insufficient for discussion at this time.

Discussion

The data are as yet incomplete in the rat "effects" on serial sacrifice group, but sufficient numbers have been studied to deserve tentative comment and comparison with other studies of strontium^{90} effects. While the measured absorbed dose had wide variation in both males and females,

the median for both sexes was essentially similar (0.71 and 0.705 μc/gm) as were the ranges of absorbed dose (male: 0.18-1.54 μc/gm; female: 0.16-1.11 μc/gm). The dose range selected has yielded results paralleling those described in mice by Finkel, Biskis, and Scribner[9] in which survivorship and the incidence of bone tumors (osteogenic sarcomas) was related to absorbed dose. For mice, significant shortening of life span did not appear below a dose level of 0.044 μc/gm, and the 50% survival figures in terms of days-after-injection of the isotope appear as follows:

controls	:	600 days
0.44 μc/gm	:	420 days
0.88 μc/gm	:	250 days
2.2 μc/gm	:	200 days
4.5 μc/gm	:	110 days

(data extrapolated from author's graphs)

The incidence of bone tumors rose sharply with increasing isotope dose, with a peak maximum of near 95% at 0.9 μc/gm. With still higher doses, the incidence fell rapidly since few animals survived long enough to develop the tumors.

Incidence of Bone Tumors vs. Isotope Dose

Sr-90 μc/gm	Incidence - %
0.04	6
0.09	4-6
0.16	17
0.4	80
0.9	95
2.0	75

The absorbed dose in our series of rats (both sexes) falls within the range of dosage resulting in greatest bone tumor incidence for mice as described by Finkel, et al. The first signs of clinical illness in the male rats developed at 140 days after exposure, but large numbers became ill between 190 and 216 days after exposures; some of these animals die

(approximately 13%) while the illness of many others was judged to be of sufficient severity to warrant unscheduled sacrifice for purpose of the planned studies.

Principal pathologic findings in the animals were marrow depletion, focal incomplete resorption of spongiosa, fibrous dysplasia, osteogenic sarcoma, and leukemia. Obvious tumors of long bones occurred in nearly one-third of the male animals, and histologic studies indicate that microscopic foci of neoplasia are present in many more. Varying degrees of fibrous dysplasia occurred, with or without frank malignant transformation. So far, approximately 20% of the male animals have developed bone tumors involving the vertebra. These have been both single and miltiple and accounted for the first clinical signs of illness (hindquarter paralysis). Animals so afflicted, developed lower extremity weakness and finally paralysis. In addition, some of these animals developed a "cord bladder" with retention and subsequent bacterial infection of the genitourinary tract.

Of particular interest was the apparent freedom from symptoms or obvious gross lesions in the parallel female group of animals, although the range of absorbed dose and median dose was essentially similar to the male group. Microscopically, a few bone tumors have been found in the females, but they have not yet been of sufficient dimension to disturb the animals, or become detectable by x-ray skeletal survey. The explanation for this difference between the two sexes receiving a similar dose at the same age is not entirely clear.

The uneven distribution of strontium is well known. Like calcium, the isotope is incorporated more rapidly in areas of active bone growth, and this feature is illustrated by the greater initial deposition found in the epiphysis of the younger animal. [20,25] This relationship between amount of local isotope concentration and rate of osteoid formation may have distinct bearing on the sex discrepancy, in view of the marked difference in growth rate between the male and female rat. Thus, higher local (epiphyseal and metaphyseal) bone metabolism in the male would probably result in a greater incorporation of isotope at such sites, with resultant enhanced

efficiency for the production of bone tumors. Using a tumorogenic dose of irradiation, the distribution of bone tumors has been correlated with the growth rate of particular bones. The incidence is higher in portions of the skeleton which have faster overall growth rates, thus accounting for the well-known pattern of occurrence of skeletal tumors.[9,20,25-28] It is also of interest that, in rats treated with hypophyseal growth hormone, the incident of tumors is higher; while in animals treated with thyroxine, the bone tumor induction period is shortened.

One may speculate upon the possibility of hormonal inhibitory or accelerative factors which might account for the sex discrepancy in the development of bone tumors. In humans, the higher natural incidence in males is well known, but a clear direct correlation with hormonal effect is rarely cited. The recently described extended survival in humans having osteogenic sarcomata treated with estrogen is suggestive of a possible direct inhibitory effect.[26]

Bone growth and development is affected by nutrition, mineral metabolic pattern, vitamins, and complex interactions of hormones particularly thyroxine, pituitary growth hormone, and steroids.[25] Estrogen appears to have a definite inhibitory effect on skeletal growth, particularly in the long bones, and tends to accelerate epiphyseal closure. Effects on mineral metabolism by acceleration of calcium and phosphorus turnover-rates has also been ascribed to estrogen effects.[9,25-27] It tentatively appears more than likely that the increase incidence of bone tumors in the male rats can be better related indirectly to sex differences in local bone metabolism and to the uptake of strontium, rather than to direct inhibitory action of estrogens or accelerative action of androgens.

Myelogenous leukemia has developed in four of the animals examined. Since less than half of the exposed rats survived the induction period for this entity (237 days), these four cases represent a significant increase in incidence as compared with the rarity of natural occurrence of this disease in this strain of rats. One of the cases was of a chronic myelocytic in type

with typical features of chloroleukemia. The other three were more immature in cell type and were, therefore, more difficult to classify. Discussions of these cases have been expanded upon elsewhere in this report. At least one of the leukemia subjects also developed an osteogenic sarcoma with pulmonary metastasis.

Figure 1 I^{131} exposed rat, age 58 days, 8 days after exposure. Autoradiographic showing deposition of isotope in thyroid gland. [X28] [I-13]

Figure 2 I^{131} exposed rat, age 66 days, 16 days after exposure. Autoradiograph showing remaining isotope deposition in thyroid gland, which completely disappeared by 32 days. [X28] [I-14]

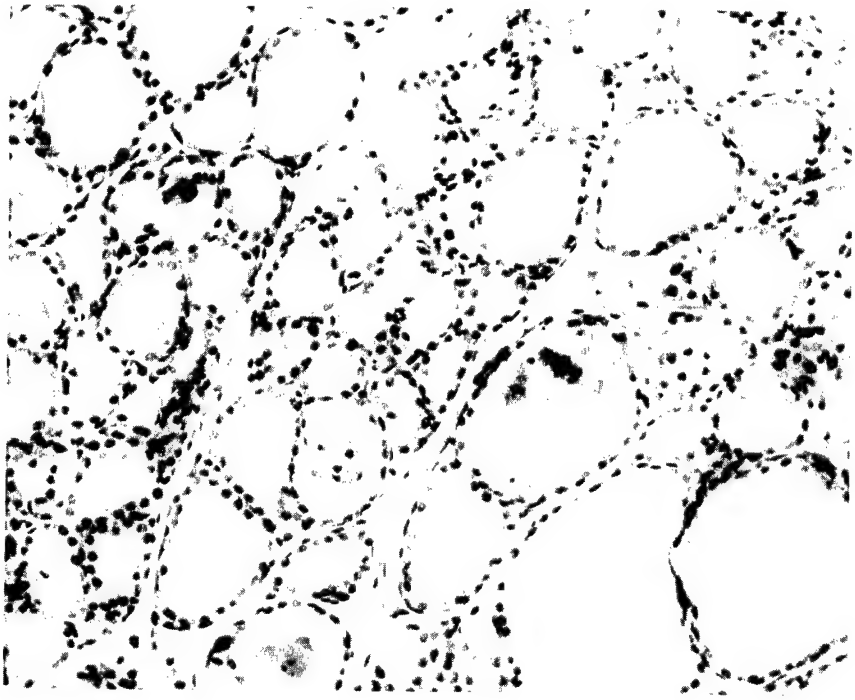

Figure 3 Control rat, age 82 days, showing usual thyroid structure. [HE] [X185] [I-14 Con.]

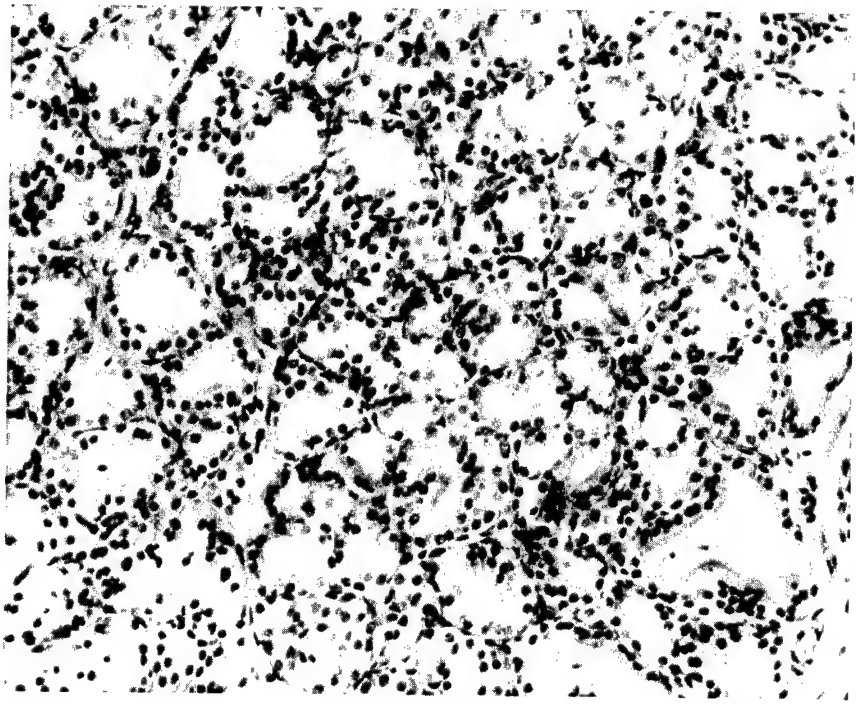

Figure 4 I^{131} exposed rat, age 82 days, 32 days after exposure, showing reduction in follicle size, paucity of colloid, and increased size of lining epithelium. [H+E] [X185] [I-14A]

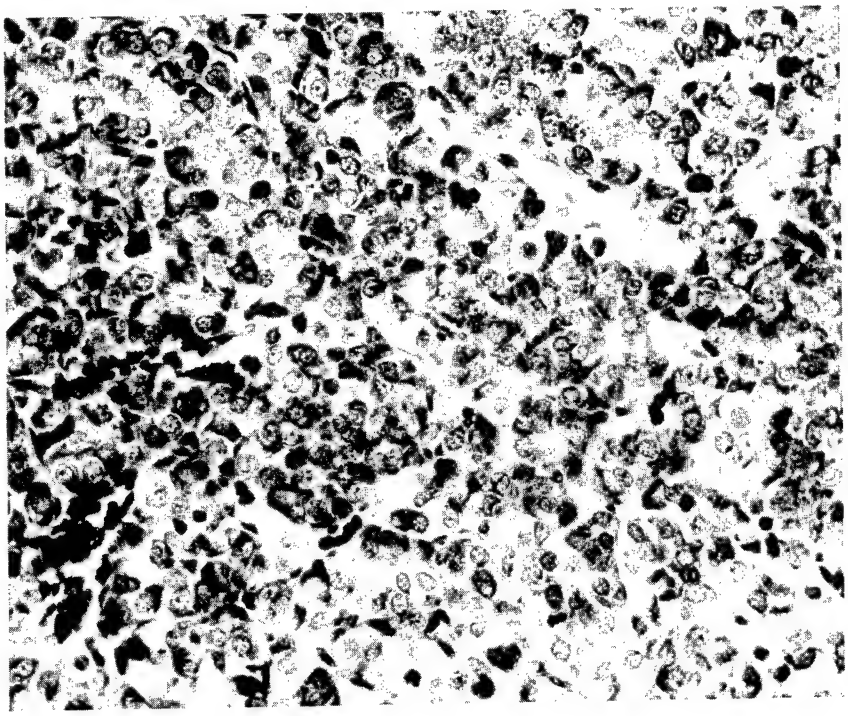

Figure 5 Control rat pituitary, age 114 days, showing usual histologic pattern of alpha cells. β-cells are quite scarce in the normal rat pituitary. [X325] [I-16 Con.]

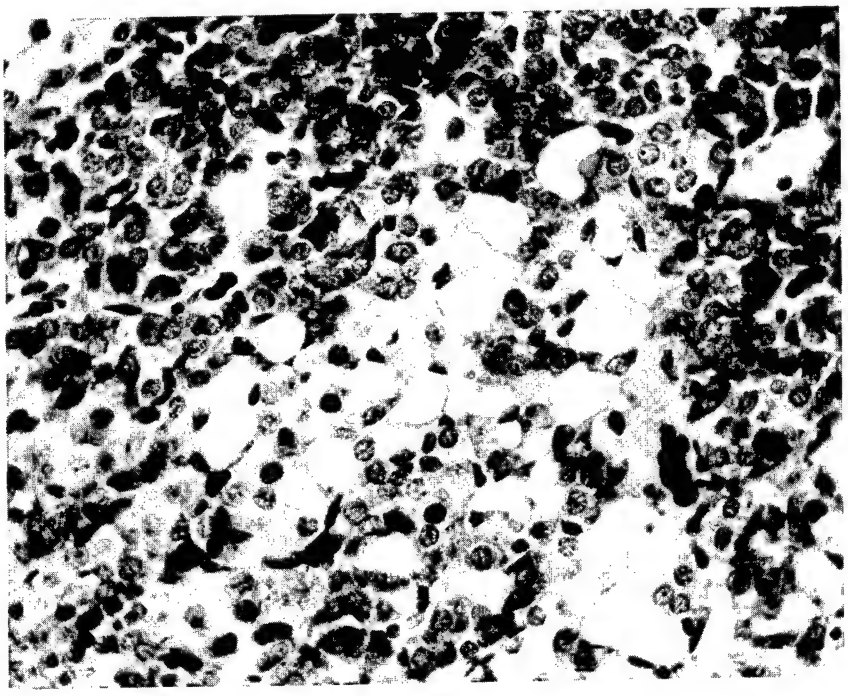

Figure 6 I^{131} exposed rat, age 114 days, 64 days after exposure, showing some degranulation of alpha cells, and frequent appearance of swollen, vacuolate Beta cells. [X325] [I-16A]

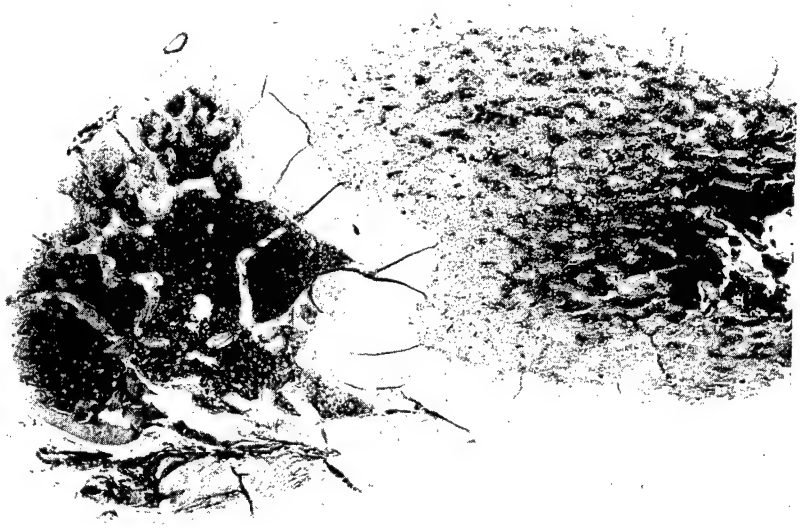

Figure 7 Section of epiphysis and metaphysis of control animal, age 46 days, showing depth of spongiosa and normal marrow hemopoietic activity. [H+E] [X14] [S-4 Con.]

Figure 8 $Sr.^{90}$ rat femur, age 58 days, 16 days after exposure, showing depletion of hematopoietic activity in metaphyseal spongiosa. [H+E] [X24] [S-5B]

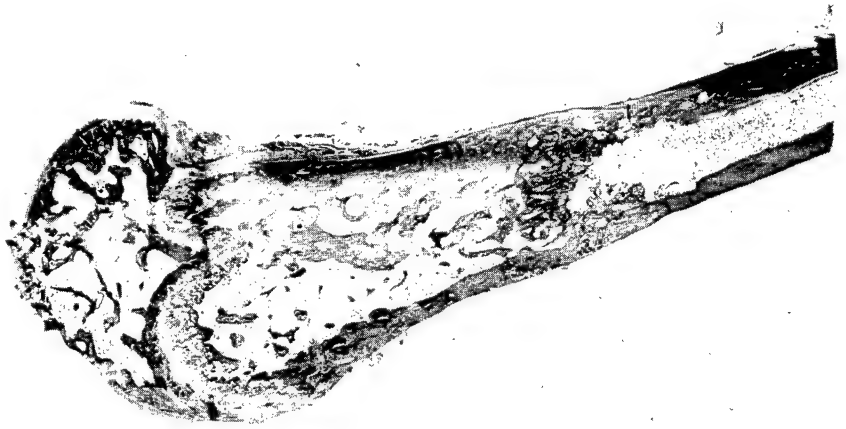

Figure 9 Sr.^{90} rat femur, age 235 days, 194 days after exposure, showing complete marrow depletion, transverse bar of unresorbed spongiosa, and extent of linear growth beyond original epiphyseal plate. [H+E] [X5.5] [S-20]

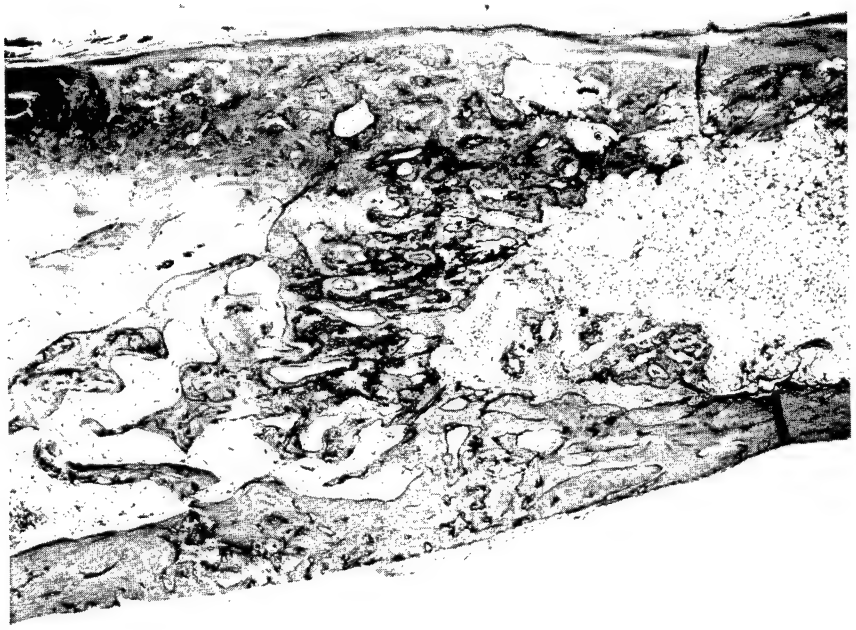

Figure 10 Sr.^{90} rat femur, age 235 days, 194 days after exposure, showing complete marrow depletion, transverse bar of unresorbed spongiosa, and extent of linear growth beyond original epiphyseal plate. [H+E] [X22] [S-20]

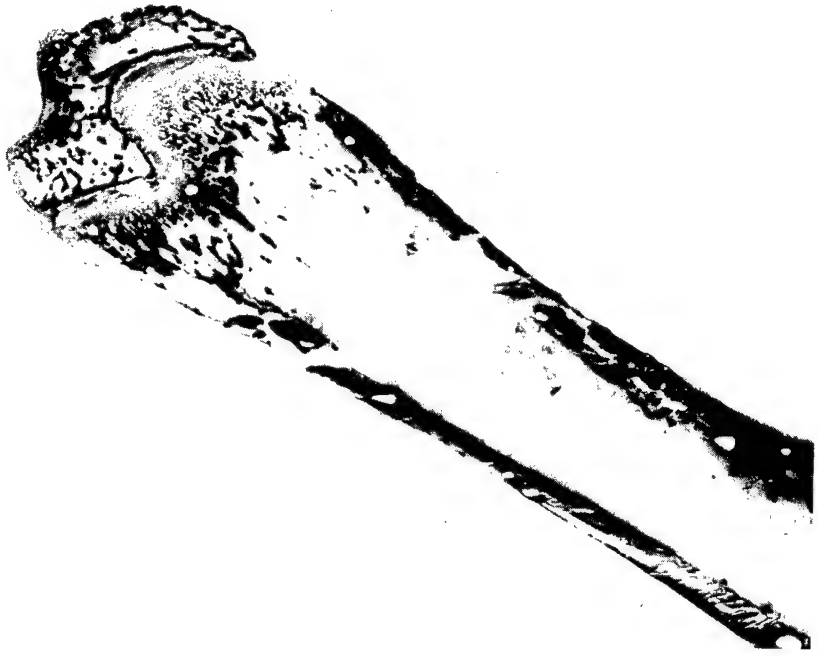

Figure 11 Femoral autoradiograph of Sr.^{90} rat, age 46 days, 7 days after exposure, showing concentrations of isotope at active mineral depositing sites of spongiosa and distribution in compact diaphysis. [X6] [S-4B]

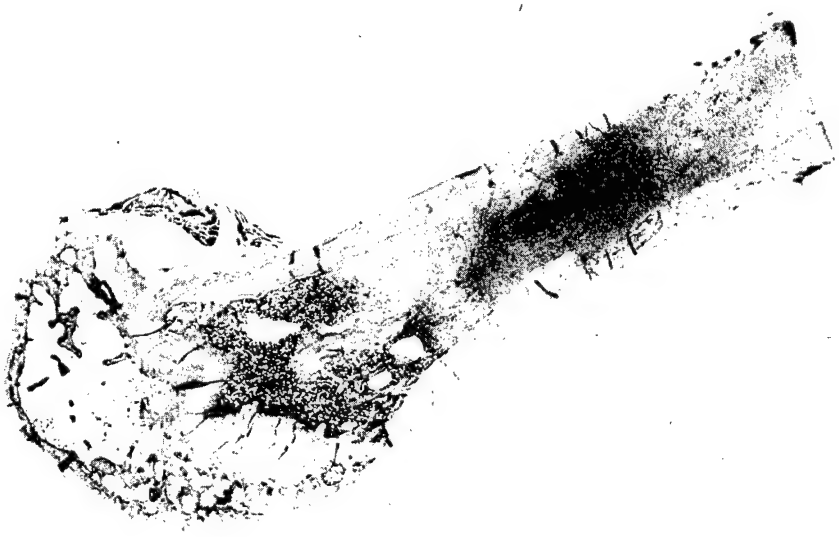

Figure 12 Sr.^{90} rat femur, age 227 days, 188 days after exposure, showing epiphysis replaced with fat, normal marrow persisting in region of metaphysis, bordered by fibrous dysplasia producing osteoid, beyond which is frank osteogenic sarcoma. [H+E] [X6-5] [S-16A]

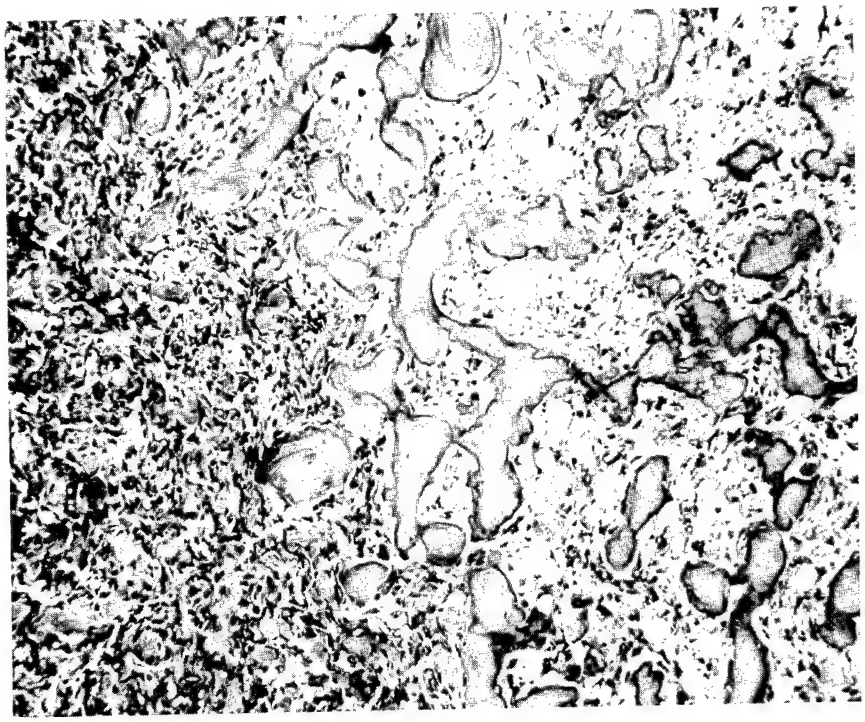

Figure 13 Sr.^{90} rat femur, higher magnification of Fig. 6 , showing transition between controversial dysplasia and fully malignant state. [H+E] [X120] [S-16A]

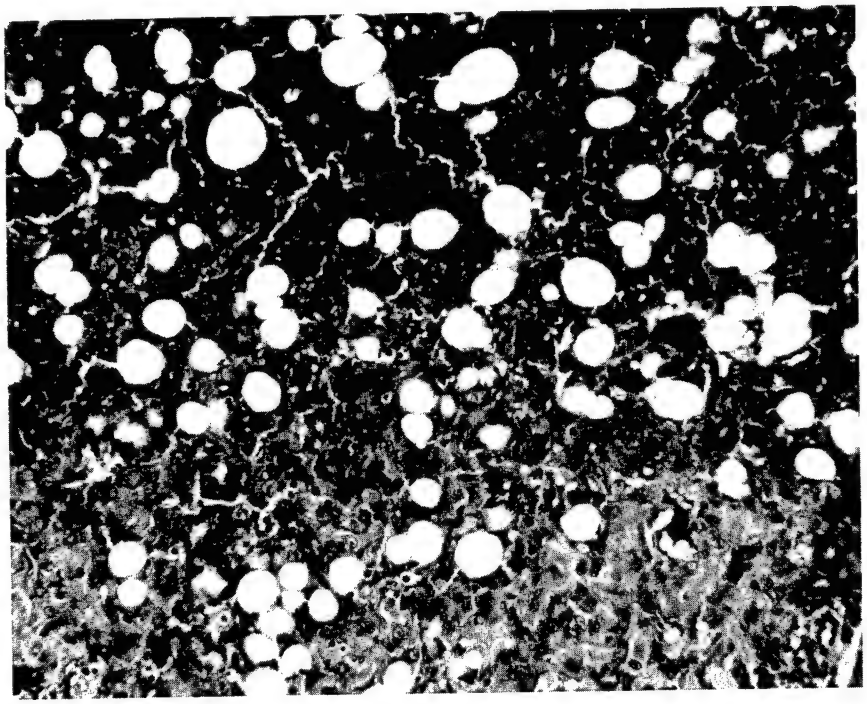

Figure 14 Sr.^{90} rat femur, higher magnification of Fig. 7 , showing marrow invasion by advancing tumor. [H+E] [X190] [S-16A]

Figure 15 Sr.^{90} rat femur, age 235 days, 194 days after exposure, showing marrow depletion, fat replacement, and irregular thickening of cortex of mid-diaphysis. [H+E] [X41] [S-20]

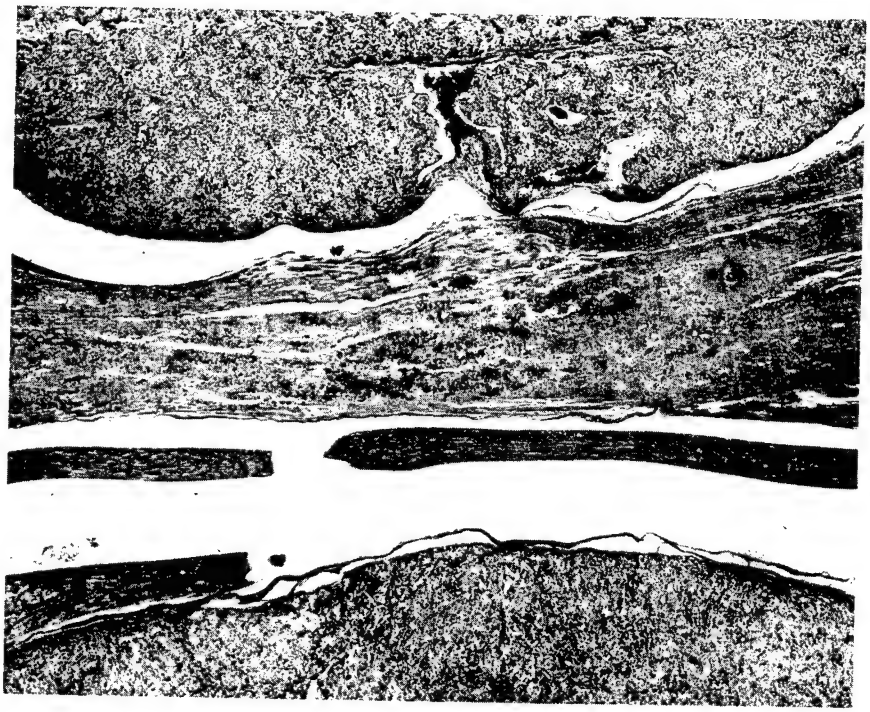

Figure 16 Sr.^{90} rat vertebra, age 229 days, 190 days after exposure, longitudinal plane section, showing spinal cord compression by bilateral masses of osteogenic sarcoma. Focal hemorrhage is present in compressed segment of cord. [H+E] [X29] [S-17A]

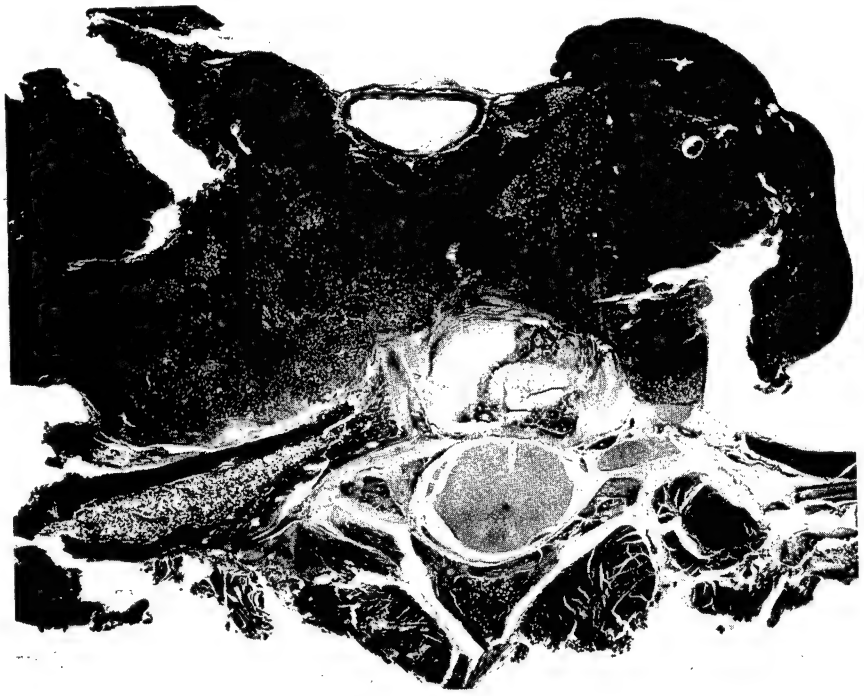

Figure 17 Sr.^{90} rat vertebra, age 233 days, 194 days after exposure, showing spinal cord and eruption of vertebral tumor mass into retroperitoneal zones and displacement of aorta. [H+E] [X7] [S-18A]

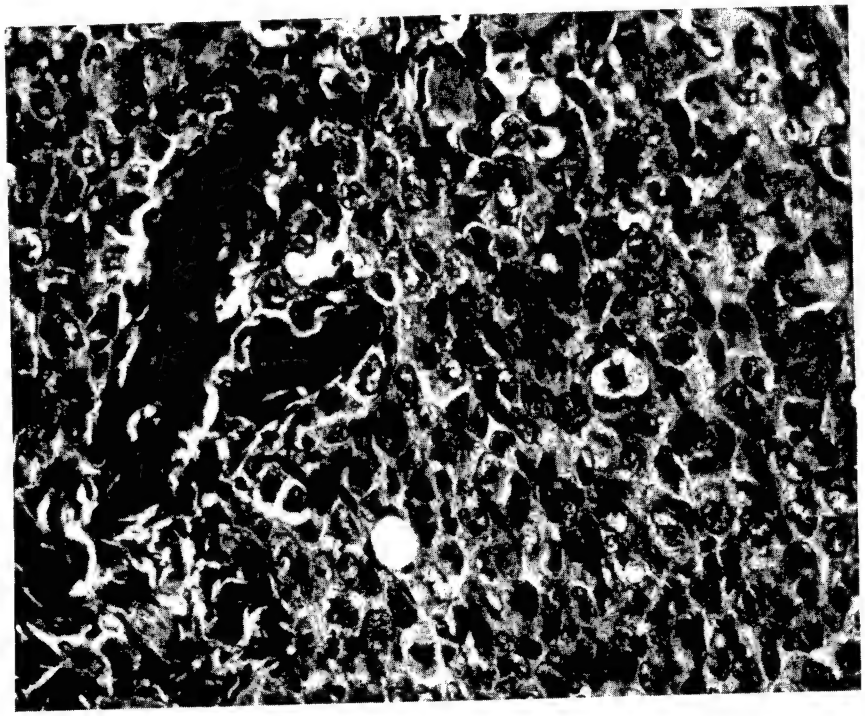

Figure 18 Sr.^{90} rat vertebral tumor, higher magnification of Fig. 11 , showing sarcomatous features and some osteoid spicules. [H+E] [X370] [S-18A]

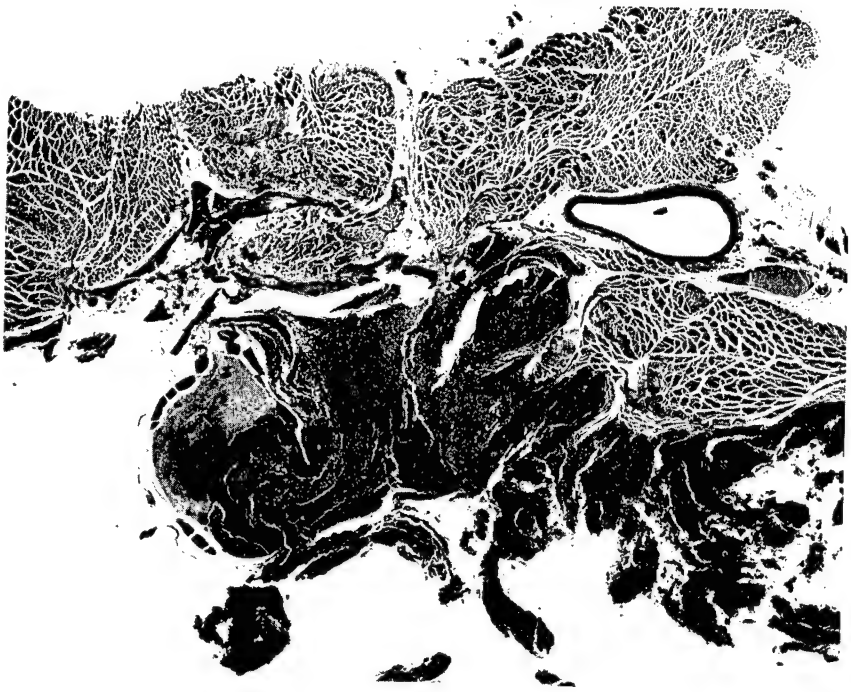

Figure 19 Sr.^{90} rat vertebra, age 270 days, 231 days after exposure, showing large tumor invading spinal cord and displacing aorta with near obliteration of vertebral bone structures. [H+E] [X9] [S-42A]

Figure 20 Sr.^{90} rat vertebral tumor, higher magnification of Fig. 13 , showing tumor invading spinal cord. [H+E] [X140] [S-42A]

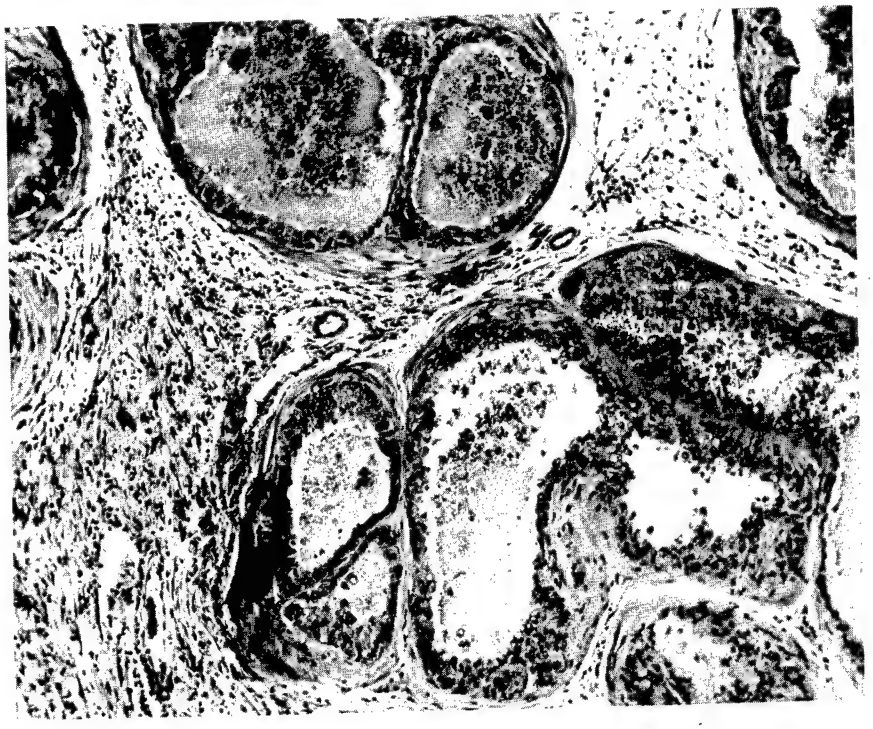

Figure 21 Sr.^{90} rat, age 235 days, 194 days after exposure, showing prostatic interstitial inflammation and fibrosis. At times, the glandular lumen is filled with purulent exudate and hemorrhage. [H+E] [X85] [S-11A]

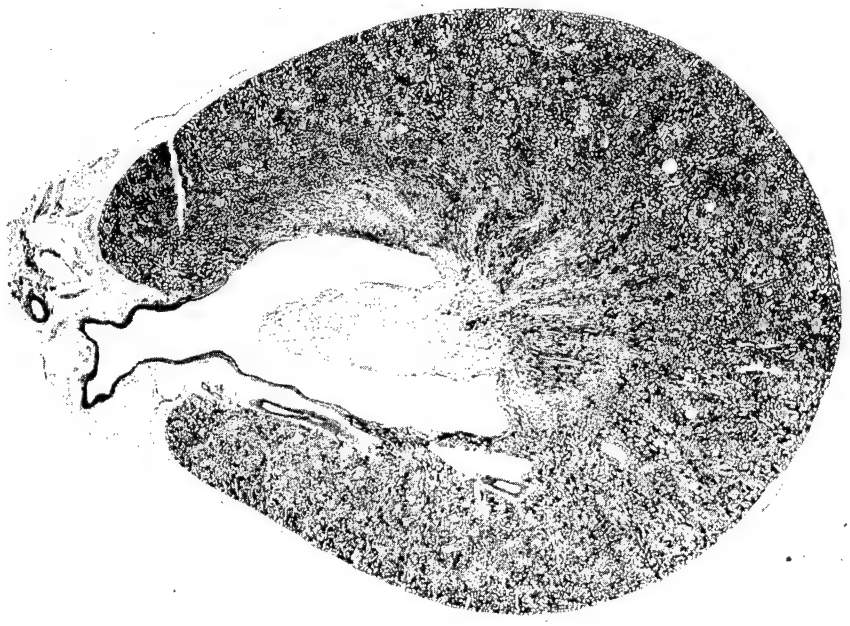

Figure 22 Sr.^{90} rat kidney, age 181 days, 141 days after exposure, showing early hydronephrotic expansion of pelvis and calyx, following partial inflammatory obstruction of distal ureter. [H+E] [X7.5] [S-11]

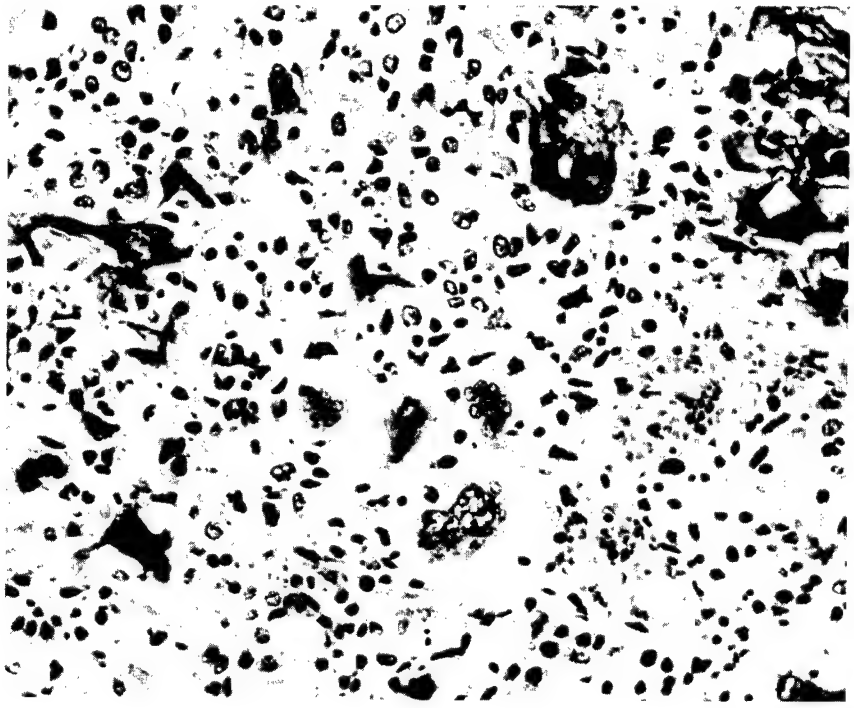

Figure 23 Sr.^{90} rat, age 242 days, 199 days after exposure, showing histologic features of pulmonary metastasis from primary bone tumor. [H+E] [X310] [S-21]

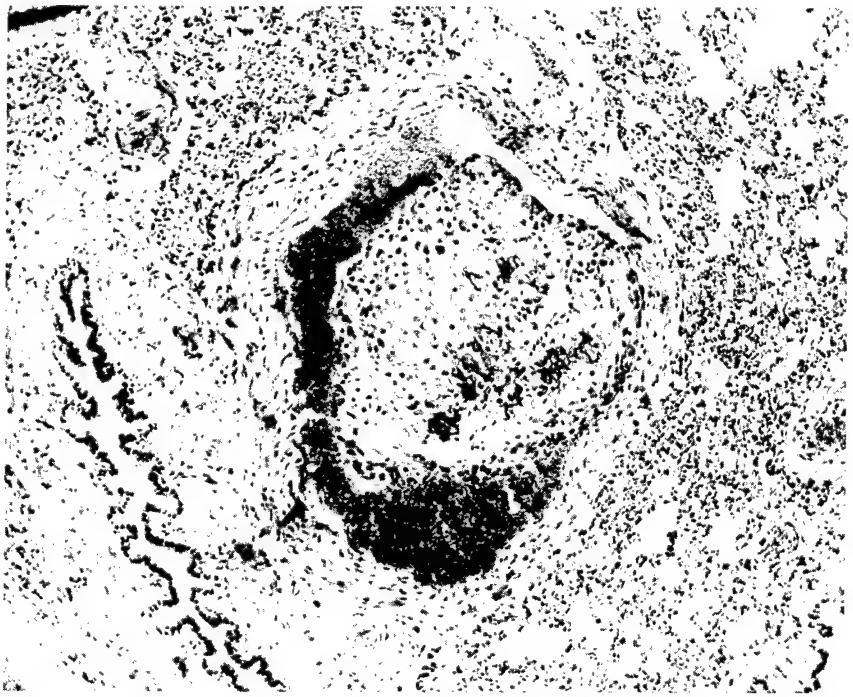

Figure 24 Sr.^{90} rat, age 235 days, 194 days after exposure, showing large tumor embolus in pulmonary artery. [H+E] [X90] [S-20]

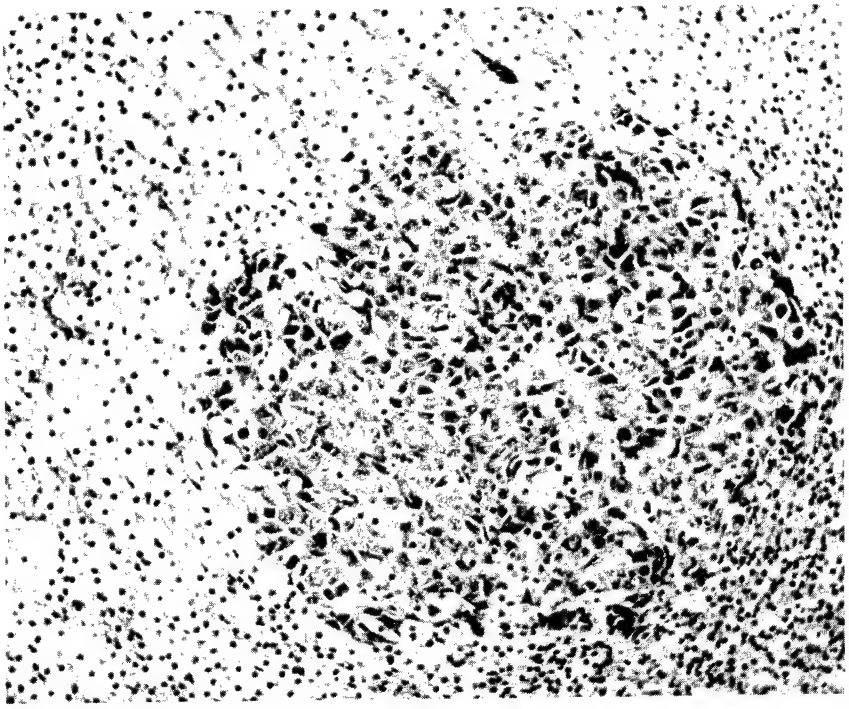

Figure 25 Sr.^{90} rat, age 248 days, 211 days after exposure, showing solitary adrenal metastasis from primary bone tumor. [HE] [X140] [S-22]

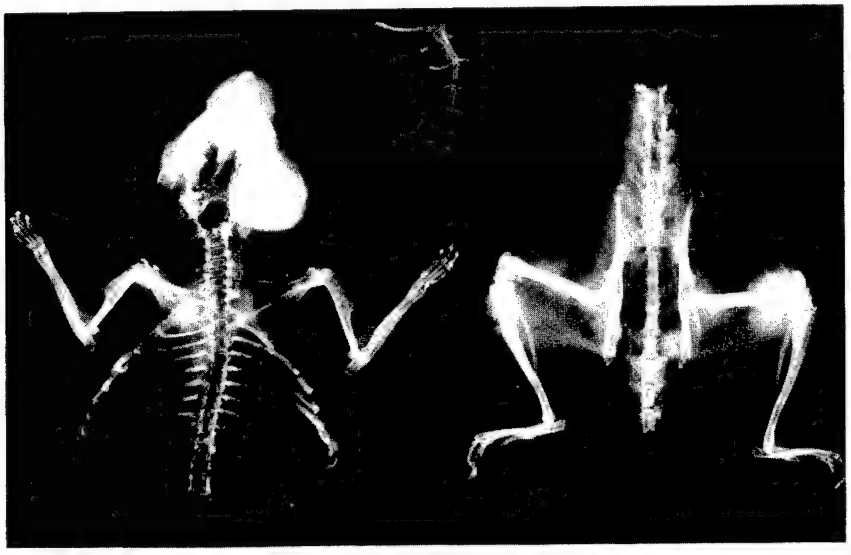

Figure 26 Sr.^{90} rat, age 305 days, 265 days after exposure. Skeletal radiograph showing large tumor masses in mandible, left upper tibia and right lower femur. [S-72A]

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EFFECTS ON BLOOD CHEMICAL CONSTITUENTS OF THE INHALATION OF I-131 OR SR-90 BY RATS AND SR-90 BY DOGS

by

E. Van Stewart and B. B. Longwell

This report presents the resulting analyses of 26 chemical constituents of blood from experimental animals used in studying the effects of internal irradiation. The radiation exposure resulted from inhalation of aerosols containing a soluble isotope. All experiments are in progress and no definitive report of biological effects can be given at this time. Studies have been conducted on the effects of I-131 and Sr-90 in rats and Sr - 90 in dogs. Ultramicro analyses have been perfected so that all 26 determinations can be made on the rat at sacrifice, and repeatedly on the dog without incurring excessive blood loss.

I. EXPERIMENTS WITH ALBINO RATS EXPOSURE TO I-131

MATERIALS AND METHODS

All of the blood chemical analyses performed following inhalation of I-131 have been on the albino rat. These rats were obtained from the Holtzman Company, Madison, Wisconsin. Some received ≈0.08 μc I-131 per gm. at an average weight of 200 gm. each at the time of exposure; some received ≈0.40 μc I-131 per gm. and ranged from 150 to 180 gms. The methods of inhalation exposure have been described.[1] Expected I - 131 body burden in the lower level group was 12 to 16 μc (total) and in the higher level group was 60 to 69 μc. Actually, the body burden varied between 2.8 and 27.2 μc in the former group and between 33.5 and 194.0 μc in the higher dose group. Hereafter, these groups will be referred to as the

0.08 μc per gm. experiment and the 0.40 μc per gm. experiment.

To obtain all of the 26 biochemical determinations desired, it was necessary to sacrifice the animals. The so-called "normal" values are, therefore, influenced by the procedures of handling (sacrificing, etc.). Not all these values can be considered true physiological normals but they can be used for comparison with results obtained from experimental groups sacrificed identically. Rats were anesthetized with sodium nembutal given intraperitoneally and as soon as the anesthesia was satisfactory (about five minutes) the abdominal aorta was exposed. The sampling needle was inserted inferior to the left renal artery and one ml. of heparinized blood was withdrawn for the determination of hemoglobin, pH, CO_2, pCO_2, HCO_3^-, acid or base excess and plasma glucose. An additional 2.5 ml. sample was collected without anticoagulant and all remaining determinations were performed on serum. These include quantification of cholesterol, total protein, protein fractions by electrophoresis, total lipid, phospholipid, total and free bilirubin, alkaline phosphatase, glutamic-oxalacetic transaminase (SGOT), glutamic-pyruvic transaminase (SGPT), lactic dehydrogenase (LDH), creatinine, phosphorus, calcium and urea nitrogen. Five control rats and five which had been exposed to I-131 were sacrificed at 1, 2, 4, 8, 16, 32 and 64 days and at sometime between 150 and 180 days. Subsequent sacrifice and sampling periods were scheduled at six month intervals after the 150-180 day period. Three surviving groups of animals remain to be sacrificed.

Ultramicro analytical procedures were used for all determinations. The general principle of the analytical system and some of the specific methods have been described.[2-5] Determinations of pH, pCO_2, total CO_2 and HCO_3^- were performed by the micro procedure of Astrup, et al.[6] with the Radiometer (Radiometer Copenhagen, Copenhagen, Denmark) pH meter. Total protein was determined by an ultramicro modification of the Kingsley[7] biuret procedure, and the electrophoretic separation of serum proteins was accomplished on cellulose acetate in barbiturate buffer at pH 8.6. Glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase and lactic dehydrogenase were determined by an ultramicro

modification of the method of Henry, et al.[8], at 32° C in a modified Beckman DU spectrophotometer equipped with the Gilford power supply (Gilford Instrument Laboratories, Inc., Oberlin, Ohio), recorder and automatic absorption cell positioner. Calcium analysis was accomplished with a complexiметric method using EDTA in the Beckman/Spinco (Beckman/Spinco, Palo Alto, California) ultramicro titrator with calcein or hydroxy naphthol blue as the indicator. Total lipid analysis utilized extraction and oxidation with potassium dichromate[9] and phospholipid was quantified by an ultramicro modification of the procedure of Connerty, et al.[10] Chloride was determined with the Cotlove Chloridometer.[11] Sodium and potassium were evaluated with the Baird, Model KY, flame photometer. No potassium determinations were done when the sample had visible hemolysis. In series B, Table 1, 20 of the 50 samples were inadvertantly contaminated and these results have been excluded.

RESULTS

A series of unexposed, control animals paralleled each experimental series. Determinations performed on the three control series were similar in every respect except the time of year at which the specimens were obtained differed. Sampling of control animals for the lower dose I-131 experiments began in February 1964 and for the higher dose, in April 1964. The first sacrifice group for the Sr-90 exposure occurred in August 1964. The biochemical data obtained from these three control groups are given in Table 1.

One observation related to the "normal" values requires comment. The results indicate that serum cholesterol increases with age, an observation that has been made on humans.[12] The mean cholesterol level for animals from the first group (1 day) up to and including the animals sacrificed at 150 to 180 days was 64 mg. per 100 ml. and ranged from 26 to 113 mg. per 100 ml. For the group of five rats sacrificed at one year, this value was 211 mg. per 100 ml. with a range of 127 to 340 mg. per 100 ml. Also, there was some indication that serum urea nitrogen increased with age. This was not as evident but the mean value was 21.1

Table 1 Blood Chemical Constituents of Male Rats*

Control Animals for Series Treated with Radioactive Isotopes

Determination	Series A Mean	Series A Range	Series B Mean	Series B Range	Series C Groups of 5 Rats	Series C Mean	Series C Range
	gm per 100 ml		gm per 100 ml			gm per 100 ml	
Hemoglobin	13.5	11.2-17.9	12.6	9.3-15.1	9	12.7	8.0-17.4
Protein, Total	6.6	5.6-7.5	6.1	4.8-7.3	12	5.6	4.2-7.3
	mg per 100 ml		mg per 100 ml			mg per 100 ml	
Glucose	180	97-291	171	93-234	13	164	84-260
Urea Nitrogen	22.2	10.2-37.8	23.5	8.1-44.4	13	20.9	10.8-34.9
Cholesterol	64.0	26-113	79.0	55-119	11	86	62-238
Total Lipids	460	211-915	436	217-674	7	358	277-482
Phospholipids	173	129-259	145	94-234	7	139	110-181
Bilirubin, Total	0.36	0.0-0.68	0.33	0.0-0.86	13	0.34	0.04-0.62
Bilirubin, Free	0.03	0.0-0.13	0.04	0.0-0.12	13	0.05	0.0-0.17
Phosphorus	7.7	4.1-11.0	8.1	4.8-12.6	12	7.8	3.3-11.9
Creatinine	0.92	0.48-1.76	0.78	0.34-1.29	13	0.92	0.31-1.46
Calcium	10.1	8.8-11.9	9.7	8.2-11.0	6	9.9	8.8-11.0
	units**		units**			units**	
Alkaline Phosphatase	47.0	22.5-87.3	70.7	24.5-143.4	12	59	26-99
	Karmen units at 32°C		Karmen units at 32°C			Karmen units at 32°C	
SGOT	156	94-372	204	73-540	12	170	76-308
SGPT	100	51-172	76	42-117	12	82	40-234
LDH	3850	1020-7415	4457	428-10940	12	2549	344-9620

Table 1 (Cont.)

Determination	Series A Mean	Series A Range	Series B Mean	Series B Range	Groups of 5 Rats	Series C Mean	Series C Range
	mEq per liter		mEq per liter				mEq per liter
Sodium	145	131-154	147	140-156	10	146	134-152
Potassium	4.7	4.0-5.5	4.6	3.6-5.6	10	4.6	3.2-5.6
Chloride	100	89-110	104	92-116	13	102	91-117
Excess H+ or OH^-	3.5 H+	0.4-7.4 H+	2.7 H+	0.1-6.4 H+	12	2.3 H+	1. OH^--7.8 H+
Actual HCO_3^-	18.4	15.1-21.3	19.4	15.4-21.9	12	19.4	16.3-24.0
pH	7.398	7.274-7.498	7.391	7.329-7.473	12	7.401	7.284-7.464
	mmHg		mmHg				mmHg
pCO_2	31.1	22.4-43.8	30.3	26.9-43.0	12	32.5	26.8-42.6
	m Moles per liter		m Moles per liter				m Moles per liter
Total CO_2	19.3	15.8-22.6	20.3	16.2-23.2	12	20.4	17.1-25.3
	Percent of Total Protein		Percent of Total Protein				Percent of Total Protein
Albumin	45.3	34.5-49.4	42.7	21.0-54.0	7	50.4	41.0-57.4
α_1 Globulin	20.7	15.1-24.4	18.3	13.9-31.0	7	16.3	11.9-22.6
α_2 Globulin	9.8	5.1-15.0	12.2	9.5-16.2	7	13.1	9.8-17.9
β Globulin	14.1	11.3-22.2	14.9	10.0-23.3	7	12.5	7.8-15.7
γ Globulin	9.4	5.4-15.2	11.1	5.7-20.7	7	7.5	3.6-15.3

*The data in the table represents mean values and range for each constituent. Fifty animals were sacrificed to obtain the data for Series A and B. The number of groups of 5 rats each examined in Series C is shown in the Table. The method of sacrifice and sampling is described in the text.

Series A served as controls for the rats which received 0.08 μc I^{131} per gm. Series B were controls for those which received 0.4 μc I^{131} per gm. Series C were control animals for those which received Sr^{90}.

**Units defined in Reference 4.

mg. per 100 ml. for all animals through the sampling period of 153 days. The range was 10.2 to 28.0 mg. per 100 ml. At the 1 year sacrifice time, five rats had a mean of 31.9 mg. per 100 ml. with a range of 26.7 to 37.8 mg. per 100 ml. Additional data will be required to establish this observation as a fact.

Results on glucose determination were influenced by the method of specimen collection and they should not exemplify "normal" glucose concentration in rat blood. Normal whole blood values for fasting rats have been reported as 66 ± 1.2 mg. per 100 ml. and 85 ± 2.0 mg. per 100 ml. for two different strains.[13] An acid excess as determined by the method of Astrup[6] was evident in all groups. These results are probably also related to the method of sampling. Respiratory depression usually results from the administration of barbiturate at anesthetic levels, and opening the abdomen must induce a shock-like response in peripheral circulation. Both of these reactions would influence results on blood acid-base measurements.

High serum activity of LDH in the rat was observed previously by Wroblewski and LaDue.[14] Glutamic-oxalacetic transaminase and SGPT are also above the values considered to be normal for human subjects.

Analyses for the same blood constituents in animals which had received 0.08 μc I-131 per gm. by inhalation revealed no values outside the ranges obtained with control animals. At first observation, when LDH and alkaline phosphatase levels of two different groups of animals were compared to their own control group, it appeared that an increase of these substances had occurred. The fallacy of this observation became apparent when the results were compared to all three control groups (Table 1). No other values were outside the ranges established for the control animals.

The findings for Series B, which served as controls for those which received 0.4 μc per gm. of I-131 are presented in Table 1. The increase of serum cholesterol with age was observed in this series as well as in Series A. A mean value of 79 with a range of 58 to 119 mg. per 100 ml.

was obtained from the animals sacrificed up to 180 days post-exposure. At the 363 days sacrifice, the group of five animals had a mean serum cholesterol of 210 with a range of 179 to 257 mg. per 100 ml. None of the animals exposed to this higher dose of I-131 demonstrated deviation from the controls in any of the blood chemical constituents analyzed. Whether this dose of I-131 is so low that it would not be expected to have had any direct effects is uncertain. Neither dose was sufficient to destroy the thyroid[15] and probably effects secondary to hypothyroidism would not be expected.

II. EXPOSURES TO SR-90

MATERIALS AND METHODS

One hundred-twenty male and forty female rats were exposed to Sr-90 Cl_2 by inhalation. The animals weighed approximately 180 gm. when exposed and they received approximately 0.5 μc of Sr-90 per gm. body weight. An equal number of male and female rats were assigned as control animals. Times of sacrifice were determined on the same basis as that described for the I-131 exposures. Blood was obtained by the same procedure described for the studies with I-131 and the analytical methods also were the same.

RESULTS

The results obtained with control male rats in this experiment are presented in Table 1, Series C. All values listed are comparable to the control determinations for Series A and B.

The animals were exposed (in groups) to Sr-90 over a time span from July 29 to August 7, 1964. Clinical evidence of possible radiation damage began to appear in the male rats in December 1964. They developed a paralysis of the hindquarters which was diagnosed histologically as a spinal cord compression syndrome due to the induction of tumors in the

lumbar vertebrae.[16] Upon sacrifice a cord involvement was noted which had resulted in so-called "cord" bladder with marked retention of urine. This radiation response was seen only in the male animals at a short, four month, interval after exposure to Sr-90. None of the female rats developed gross symptoms at this early period. Three months later, a total of seven months after exposure to the isotope, it was decided to begin sacrificing the females which had originally been intended for a longevity study. Even at seven months these females had not developed gross symptoms. Female control animals were sacrificed at the same time; values for their blood analyses are recorded in Table 2. Some of the determinations have not been completed at this time, but the results for completed analyses are of the same order as those obtained with male animals. Beginning at the seventh month, the sampling schedule was changed so that a group of five males and five females, together with their respective controls, were sacrificed each month.

Analysis of the blood chemical constituents of those rats which showed gross evidence of radiation effects showed certain deviations from the control values. Most of these changes can be related to the effect of the cord bladder on urine flow. The results obtained with 15 male rats, all of which demonstrated gross radiation effects and had proven sarcoma, are listed in Table 3. The analyses have not been completed at this time and it is possible that additional changes may be noted when the study is terminated.

DISCUSSION

No changes have been found in the level of the measured blood chemical constituents, as the result of the administration of I-131 at either 0.08 μc per gm. or 0.40 μc per gm. These are both relatively low doses of a short half-life isotope. The doses caused no apparent direct effects and it is evident that administration by the pulmonary route does not result in reactions other than might be expected from administration by any other route. Effects which might have been secondary to thyroid damage are also unlikely, according to the reports of Goldberg, et al.[15] These authors

Table 2 Blood Chemical Constituents of Female Rats*

Control Animals for Series Treated with Sr^{90}

		Series D	
Determination	Groups of 5 Animals	Mean	Range
		gm per 100 ml	
Total Protein	3	6.5	5.0-8.7
		mg per 100 ml	
Glucose	4	131	90-260
Urea Nitrogen	4	24.3	19.8-33.9
Bilirubin, Total	4	0.34	0.09-0.61
Bilirubin, Free	4	0.07	0.0-0.15
Phosphorus	3	5.7	3.0-6.8
Calcium	4	9.9	9.7-10.5
Creatinine	4	1.1	0.75-1.43
		units	
Alkaline Phosphatase	3	33.0	21-67
		Karmen units at 32°C	
SGOT	3	160	89-426
SGPT	3	115	89-157
LDH	3	1446	368-8316
		mEq per liter	
Chloride	4	106	84-114

*The collection of blood from these animals began 7 months after the experimental female series had been exposed to Sr^{90}. See text.

Table 3 Blood Chemical Constituents of Male Rats Treated with Sr^{90}*

Rat No.	Day Post Exp.	Hemo-globin	Total Protein	Urea Nitrogen	Creatinine	Total Bilirubin	Calcium	pH	Acid Excess	pCO_2	SGOT	Alkaline Phosphatase
		gm per 100 ml		mg per 100 ml					mEq per l	mmHg	units**	units**
1	141	2.4	5.9	260.0	6.3	0.35	6.4	6.915	22.8	40.4	258	20.9
2	147	9.8	5.9	21.8	-	4.54	10.4	7.305	6.6	38.0	528	77.6
3	190	11.3	5.0	38.0	1.0	0.33	9.9	7.286	6.1	40.4	128	54.0
4	192	17.3	5.8	48.0	1.1	0.20	9.3	7.477	4.2	22.0	159	8.5
5	194	13.1	-	41.0	1.3	0.30	9.4	7.338	5.4	35.0	187	13.0
6	199	14.4	6.3	46.0	-	0.43	9.9	7.178	10.5	46.7	267	-
7	210	15.2	5.1	25.6	1.0	0.27	10.0	7.278	1.4	54.0	183	-
8	212	16.3	5.8	55.0	1.2	0.18	8.5	7.393	4.6	29.5	169	39.0
9	213	15.4	5.7	105.0	1.1	0.18	9.0	7.348	8.0	28.0	262	40.0
10	220	15.6	-	-	-	0.12	10.5	7.223	5.3	55.3	188	-
11	221	16.5	4.4	67.0	1.2	0.20	9.7	7.348	4.8	34.6	297	62.0
12	235	13.5	3.8	25.0	1.0	0.15	9.5	7.447	4.6	24.8	85	31.0
13	238	12.8	4.3	83.0	1.0	0.08	9.0	7.387	6.7	26.8	195	35.0
14	238	2.7	4.6	84.0	1.0	0.68	9.5	7.362	11.3	22.0	1060	24.0
15	244	13.1	5.9	91.0	1.4	0.10	10.0	7.385	8.7	23.0	223	-

*The blood was obtained from animals which had developed gross evidence of radiation effects after inhalation of Sr^{90}. The underlined values are outside the limits found in the control series and only those constituents are listed for which one or more animals showed an abnormal value for the substance.

**See definition in Table 1.

administered doses of approximately 2.1 and 3.5 μc I-131 per gm. to rats by parenteral injection. Some epithelial regeneration of the damaged thyroid gland occurred at the lower dose, none at the higher one. The dose administered in the present experiments was far too small to result in ablation of the thyroid.

The short time required for the appearance of tumors in the male rats compared to the females which had received Sr-90 was an unexpected development. Gross evidence of this effect of Sr-90 first appeared in male rats approximately four months after the isotope was administered. Bone tumors were observed for the first time in the female rats which were sacrificed approximately 226 days after exposure. A description of the tumors, their distribution and metastatic spread, is presented in this report by the Department of Pathology.[16]

The deviation from control values of the blood substances shown in Table 3 are most likely secondary to the effects of the tumor growth and spread. They should not be interpreted as direct radiation effects. Elevation of urea nitrogen is the result of cord bladder with consequent interference with urine flow. Mild hydronephrosis resulting from the retention of urine was observed in some animals. Creatinine retention was also evident in animal 1, the rat with the most pronounced retention of urea. Three of the rats, numbers 1, 6 and 10 (Table 3) had blood pH less than the lowest found in the control series. Five of them, numbers 1, 6, 9, 14 and 15 were in a state of metabolic acidosis considerably greater than any in the control series, as measured by an acid excess. These findings probably result from the interference with urine flow. The estimated pCO_2 was elevated above the control series values in rats number 6, 7 and 10. Metastatic growth of tumor in the lungs,[16] with associated pneumonic and pneumonitis changes are the likely causes of these increases.

The decrease in serum calcium and hemoglogin in animal 1 and the hemoglobin in animal 14 may be related either to kidney damage, or to a direct effect of the Sr-90 on bone marrow. A reduction in total serum protein occurred in animals 11, 12, 13 and 14. This, too, should probably be ascribed to renal damage, although anorexia may also have been

a causitive factor.

Less readily related to the observed effects of the tumors on the urinary tract and lungs is the elevated SGOT found in rats 2 and 14. No liver involvement was evident to account for these increased levels of activity, but the serum from rat 2 also had an increased bilirubin concentration.

Finally, serum alkaline phosphatase had decreased significantly in animals 1, 4 and 5. Unpublished experiments in this laboratory have demonstrated that withholding food will cause a fairly rapid and progressive drop in serum alkaline phosphatase in the rat. It seems likely that anorexia was responsible for the decrease observed in these animals which had received Sr-90.

III. EXPERIMENTS WITH DOGS

Biochemical experimentation on beagles was initiated in November 1964 and to date, 24 dogs have been exposed for this purpose. Also, a considerable amount of data have been accumulated to evaluate the normal concentration of various blood chemical constituents in beagles of both sexes. These data, together with a brief summary of the radioactive aerosol exposure studies done thus far, are the subject of the following portion of this report.

MATERIALS AND METHODS

Dogs 12 to 14 months of age were used both in the evaluation of normal blood concentration of various chemical substances and in the inhalation exposure to soluble Sr-90 Cl_2. Blood specimens were taken from the jugular vein and the femoral artery of unanesthetized dogs. Arterial blood was collected in a heparinized syringe for the determination of pH, PCO_2, total CO_2 and HCO_3^- by the methods of Astrup, et al.[6] Glucose was determined in plasma obtained from the arterial specimen. Venous blood was collected without an anticoagulant. The remainder of the constituents considered were quantified in serum. Blood samples were collected in

the morning about 1 hour before the usual feeding time and therefore the animals had been fasted approximately 24 hours before sample collection.

Ultramicro analytical procedures were used throughout, in order to permit repeated sample collection without introduction of problems related to excessive blood loss. The analytical methods were the same as those described previously for the experiments using rats.

The 24 dogs exposed to Sr-90 were comprised of 14 males and 10 females. Three males and 6 females served as parallel controls. The dose level sought was 125 μc Sr-90 per kilogram, but because of the uncertainties involved in inhalation studies the dose actually ranged from 61 to 200 μc per kilogram. Pre-exposure blood samples were obtained on all of the animals before they received the radioactive isotope. Two venous specimens were collected, one at 2 weeks and one at 1 week before isotope treatment, and one arterial specimen was taken 1 week prior to exposure. The first portion of this group consisted of 7 male experimental dogs and 3 female controls. Certain dogs of this first group were sacrificed according to the following plan:

Blood samples taken at:

4 days
5 days (2 exposed, 1 control sacrificed)
1 week
2 weeks
4 weeks (2 exposed, 1 control sacrificed)

Blood specimens were collected from the remaining 3 exposed dogs and 1 control at post-exposure times of 5, 7, 9 and 11 weeks and monthly thereafter until June 1965. Specimens will be taken at 6 month intervals hereafter until the animals are sacrificed.

The remaining animals in this first "effects" study, 7 males and 10 females, received Sr-90 (ten minute exposure period) sometime between February and June of 1965. Blood specimens were taken before exposure and at approximate intervals of 2, 5 and 12 weeks after exposure and additional specimens will be obtained at 6 month intervals until death or planned sacrifice supervenes. Three male and 3 female unexposed, control dogs are being maintained parallel to the exposed, experimental animals.

RESULTS

The normal values for blood chemical constituents of male and female beagles, 12 to 14 months of age, are recorded in Table 4. These results are incomplete because an equipment malfunction has necessitated the postponement of the sodium and potassium analyses. These values will be reported at a later date. Electrophoretic separations of serum proteins are not sufficient in number to justify reporting results at this time.

Table 5 records the results obtained on two dogs, number 2, a male experimental animal exposed to Sr-90 and number 3, a female control animal. These samples were obtained over a 172 day period from the time of exposure of number 2. The data are presented for the purpose of showing the ranges of values to be expected for each determination in a single animal over an extended period of time.

To date only one of the exposed animals has shown any variation from the normal ranges of blood chemical constituents. The exception is one female which received 128 μc of Sr-90 per kilogram. Twenty-nine days later this animal developed a fever, was clinically in a critical state and was sacrificed two days later. Significant increased concentrations of cholesterol (490 mg. per 100 ml.) and alkaline phosphatase (16.6 units) were found. Post-mortem examination showed no change which could be correlated definitely with these findings.

DISCUSSION

Most of the values in the normal group, Table 4, are of the same order as the values found for the same constituents in normal human blood. One exception is the level of lactic dehydrogenase activity. In the beagles, as in the rat, considerably more activity is found than is present in human blood. A high level of LDH was also observed in mongrel dogs by Highman and Altland.[17]

Normal serum cholesterol in females appears from the concentrations listed in Table 4 to be higher than in males, if judged solely by the mean

Table 4 Normal Values of Some Blood Constituents of Young Beagle Dogs*

Determination	Number of Dogs Sampled	Mean	Range
		gm per 100 ml	
Total Protein	11 ♂	6.2	5.7-7.1
	14 ♀	6.3	5.2-6.9
		mg per 100 ml	
Cholesterol	7 ♂	181.0	142-226
	7 ♀	234.0	151-415
Glucose	12 ♂	101.0	89-117
	15 ♀	98.0	67-117
Bilirubin, Free	12 ♂	0.03	0.01-0.07
	15 ♀	0.05	0.0-0.13
Bilirubin, Total	12 ♂	0.21	0.15-0.27
	14 ♀	0.24	0.15-0.38
Calcium	12 ♂	11.0	10.3-11.7
	15 ♀	11.1	10.6-11.7
Creatinine	12 ♂	0.93	0.70-1.3
	15 ♀	0.90	0.70-1.3
Phosphorus	9 ♂	4.3	3.6-5.1
	13 ♀	4.6	3.8-5.6
Urea Nitrogen	12 ♂	11.6	9.2-15.0
	15 ♀	11.4	8.6-16.0
		units	
Alkaline Phosphatase	9 ♂	2.7	1.9-3.5
	12 ♀	2.9	1.6-4.3
		mmHg	
pCO_2	12 ♂	28.0	23.4-34.0
	15 ♀	28.2	23.5-33.0
		m Moles per liter	
Total CO_2	12 ♂	17.4	13.9-19.5
	15 ♀	18.2	16.5-20.7

Table 4 (cont.)

Determination	Number of Dogs Sampled	Mean	Range
		Karmen units at 32°C	
SGOT	11 ♂	39.4	28.0-54.0
	14 ♀	40.2	16.0-54.0
SGPT	12 ♂	80.5	70-92
	15 ♀	79.4	34-199
LDH	11 ♂	564	224-1186
	14 ♀	487	182-856
		mEq per liter	
Excess Acid or Base	12 ♂	5.1H+	3.3-9.7H+
	15 ♀	4.2H+	0.OH^--6.4H+
Actual HCO_3^-	12 ♂	17.0	13.2-18.7
	15 ♀	17.6	16.0-20.0
Chloride	12 ♂	111	103-120
	14 ♀	113	103-120
pH	12 ♂	7.404	7.347-7.462
	15 ♀	7.415	7.364-7.531

*The blood specimens for these analyses were taken after an approximate 24 hour fast. Heparinized arterial blood (femoral artery) was used for the determination of pH, pCO_2, HCO_3^-, total CO_2 and plasma glucose. Blood was also drawn from the jugular vein without anticoagulant and the remaining determinations were performed on serum.

Table 5 Blood Chemical Constituents of a Normal Female Beagle and of a Male Beagle Exposed to Sr^{90} by Inhalation*

	Normal Female			Male Treated with Sr^{90}		
Determination	Number of Samples	Mean	Range	Number of Samples	Mean	Range
		gm per 100 ml			gm per 100 ml	
Total Protein	13	6.0	4.5-7.1	12	5.9	5.0-6.4
		mg per 100 ml			mg per 100 ml	
Glucose	13	97	78-128	14	97	64-150
Cholesterol	11	188	98-272	11	151	125-172
Bilirubin, Total	14	0.23	0.17-0.33	14	0.25	0.12-0.35
Bilirubin, Free	13	0.04	0.0-0.12	13	0.09	0.0-0.28
Urea Nitrogen	12	10.5	8.4-12.5	12	10.0	7.5-12.8
Creatinine	12	1.00	0.87-1.22	12	0.92	0.76-1.23
Phosphorus	12	4.2	2.8-4.7	12	4.0	3.5-4.7
Calcium	12	11.5	10.3-12.8	12	11.2	10.4-12.5
		units**			units**	
Alkaline Phosphatase	13	3.0	2.0-4.5	13	2.1	0.4-3.5
		Karmen units at 32°C			Karmen units at 32°C	
SGOT	13	39	27-50	12	37	26-67
SGPT	13	64	45-95	12	74	56-103

Table 5 (Cont.)

Determination	Normal Female			Male Treated with Sr^{90}		
	Number of Samples	Mean	Range	Number of Samples	Mean	Range
		mEq per liter			mEq per liter	
Sodium	5	148	142-152	4	146	143-150
Potassium	5	4.5	4.1-4.9	4	4.5	4.4-4.6
Chloride	12	110	105-118	11	113	108-125
Actual HCO_3^-	12	17.5	14.6-19.2	12	18.5	15.2-20.5
Excess Acid or Base	12	3.8 H+	6.5-0.9 H+	12	2.5 H+	0.4-6.7 H+
pH	12	7.424	7.402-7.445	12	7.443	7.399-7.464
		mmHg			mmHg	
pCO_2	12	27.6	25.2-30.0	12	27.9	24.4-31.0
		m Moles per liter			m Moles per liter	
Total CO_2	12	18.3	15.3-20.1	12	19.3	15.9-21.4

*Blood specimens were taken one week prior to exposure to Sr^{90}, and at intervals of 4 days, 1, 2, 4, 5, 7, 9 and 11 weeks and monthly thereafter for 5 months.

**See Table 1 for definition.

values. However, a single female had a cholesterol level recorded at 415 mg. per 100 ml. but the next highest one was 280. Only seven specimens were analyzed and when more determinations have been added to this group the apparent difference between male and female may not be seen.

Most of the control animals analyzed showed a mild, compensated metabolic acidosis. The 24 hour fast prior to blood collection is probably responsible for this observation.

In the animals observed to date, with the one exception noted in the Results, no deviation from normal ranges of blood chemical constituents have been encountered. A considerable number of dogs will be examined eventually over the course of a long experimental period. Any changes which may be related either to aging or to the effects of Sr-90 will eventually be documented.

SUMMARY

Albino rats and Beagle dogs have been exposed by inhalation to aerosolized radioactive isotope preparations, and blood has been analyzed for 26 constituents at intervals after the exposure. Rats received I-131 at two dose levels and Sr-90 at a single dose level. Dogs received Sr-90 at one dose level, only.

Ultramicro quantification of blood constituents showed no deviations from the values of the unexposed, control animals in the I-131 experiments with rats. However, male rats which received Sr-90 developed osteogenic neoplasms, some of which were located in the vertabrae causing cord compression syndrome. Among these were several which developed "cord bladder" with blood chemical changes that attributed to interference with urine flow.

No deviations from normal have been observed to date in the blood of dogs exposed to Sr-90.

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ISOLATION OF VIRUSES FROM BEAGLES EXPOSED TO AN AEROSOL OF SR-90

by

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ABSTRACT

Eight beagles exposed by inhalation to sublethal amounts of Sr-90 and eight controls were sampled by nose, throat and rectal swabs for the isolation of viruses. Five hundred and eight specimens were taken in the first 5 months. All specimens but 12 from the 3 sources have been passed 3 times through monkey kidney cell cultures, most through HeLa cells and approximately one-half through canine tissue cultures. Forty-three agents were isolated in MK cells and 99 from HeLa cells but none from dog tissue cultures. Only nine of these agents have been tentatively identified. The nine appear to be Coxsackie B1 virus. No significant differences at this point have been observed between the control and experimental group.

INTRODUCTION

As part of a program to determine possible changes associated with exposure to inhaled fission products, an attempt was made to isolate and identify any viruses present in the nose, throat or feces of exposed and non-exposed beagles. The following is a report of findings during the first 5 months following exposure to 31-46 microcuries of Sr-90.

METHODS

The 16 dogs used in the previous experiment for bacteriological

studies were used for virus isolations (see Clapper et al., this report). Swabs were taken and processed in the same manner as described by Pindak and Clapper.[1] The nose, throat and rectal swabs were taken before exposure to the radioactive aerosol and at 6, 12-13, 19-20, 26-34, 40-48 days and at monthly intervals thereafter for 11 months. Results on only those specimens taken during the first 5 months are reported here as data on the remainder are incomplete or lacking.

Specimens were inoculated into 2 tubes each of primary monkey kidney cells (MK), HeLa cells and one type of primary cell culture derived from dog tissue. These latter cultures were dog lung or trachea in most cases. Whether the cultures showed a cytopathic effect from the inoculum or not, they were passed at least three times in the kind of cells first inoculated.

For purposes of economy, nose and throat specimens were combined in equal parts for inoculation into the MK cells. In all other cell cultures they were inoculated separately. All of the MK cultures that were negative for cytopathic effect after the first passage were tested with guinea pig red blood cells for hemagglutinating activity. Such tests were done to detect myxoviruses. All 3 types of cultures which were negative after the 3rd passage were tested with guinea pig, human, chick and dog erythrocytes for hemagglutinating activity. The results of these tests are not complete and will be reported later.

RESULTS

Table 1 summarizes the work completed on specimens taken during the first 5 months. Although nasal, throat and rectal swabs were taken at monthly intervals 5 more times no initial inoculations have been done with them. Table 1 indicates that by combining nose and throat specimens for the MK cells a total of 336 specimens will have to be passed 3 times in these cultures to complete the work; 324 have been completed. In the HeLa cells 508 specimens will have to be passed and of these 412

Table 1

Isolation of Viruses from Dogs

Summary of Work Completed on Specimens Collected During the First 5 Months

	Primary Monkey Kidney			HeLa			Primary Dog Lung			Dog Trachea		
	Passage			Passage			Passage			Passage		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Number of Specimens	336	336	336	508	508	508	508	508	508	508	508	508
Completed	336	336	324	508	461	412	268	268	187	166	107	105
Not Completed	0	0	12	0	47	96	240	240	321	342	401	403
Total of Completed Inoculations in all Passages	996			1381			723			378		
Total of Inoculations to be Completed	12			143			801			1146		
Number of Isolations to Date	43			99			0			0		

have been completed in the 3rd passage. Five hundred and eight specimens would also have to be passed through dog lung and trachea tissue cultures if all were to be so processed. Completed passages have been made in dog lung with 187 specimens and with trachea in 105. Since no isolations have been made in either of these cell lines it is felt that adequate coverage will be given by passing the remainder through dog lung only. Our previous experience[1] with dog kidney yielded no positive results although several agents were found in MK and HeLa cells. Unless hemagglutinating reactions can be shown in the inoculated canine tissue cultures, it appears that the dog's own cells prepared under these conditions[1] are not useful for isolating viruses from dogs. The only ones thus far reported are infectious canine hepatitis,[2,3] adenovirus[3] and reovirus.[4] The most commonly isolated virus in dog kidney has been infectious canine hepatitis and the beagles in this study were immunized against it, and have had no contact with non-immunized dogs.

There have been 43 agents which caused cytopathic effects isolated from the MK cells, and 99 from HeLa cells (Table 1). Nine of these isolates have been neutralized by Coxsackie B1 antiserum; antiserum made against one of these isolates neutralized the 9 isolates and the prototype Coxsackie B1 which is maintained in this laboratory. In one dog a cytopathic agent was isolated from rectal and nose swabs taken both before and after exposure. Identification of the remaining agents has not been made.

There does not appear to be any difference in the number of isolations made from the control dogs and those that were exposed to the aerosol (Table 2). This finding may have been expected because at this dose of Sr-90 no clinical effects are observed. However, the fact that the dogs harbor viruses may be important clinically in future experiments with higher doses of radiation should the latter significantly lower the resistance of the animals.

Table 2

Isolation of Viruses from Nasal, Throat and Rectal Specimens

	Days After Exposure																					
Dog No.	Before Exposure		6		12-13		19-20		26-34		40-48		55-62		68-69		88-103		123-238	
Exptls.	N+T	R	N+T	R	N+T	R	N+T	R	N+T	R	N+T	R	N+T	R	N+T	R	N+T	R	N+T	R
FD-13	+	+	+	+	+	+	0	0	0	+	0	0	0	0	+	0	0	+	0	0
FD-17	+	+	+	+	+	+	+	0	+	+	0	0	0	0	+	+	0	+	+	0
FD-23	0	+	+	+	0	0	+	0	+	+	0	0	0	0	+	+	0	0	0	0
FD-82	0	+	+	+	+	0	+	0	0	+	0	0	0	0	+	+	0	0	0	0
1C	+	+	0	0	+	0	0	+	0	0	+	0	+	+			0	0	+	0
FD-14	0	+	0	3	0	0	0	+	0	0	0	+	0	0			0	0	0	0
FD-19	0	0	0	+	+	0	0	0	0	+	+	0	+	+			0	0	0	0
FD-83	+	+	+	0	+	0	0	0	+	0	+	0	+	+			+	0	+	0
Total Spec. Positive	4	7	5	5	6	2	3	2	3	5	3	1	3	3	4	3	1	2	4	1
Total Dogs Positive	7		6		6		5		6		4		3		4		3		4	
Controls																				
FD-88	+	0	+	+	0	+	0	0	0	+	+	0	0	0	+	+	0	+	0	0
1E	+	+	+	+	0	+	0	0	+	+	0	0	0	0	0	0	0	0	0	0
FD-84	+	+	0	+	0	0	0	0	0	+	0	0	+	0	0	+	0	+	0	0
FD-24	0	0	+	0	0	0	0	0	+	+	0	0	0	0	+	+	0	0	0	0
1D	0	+	0	0	0	0	+	+	+	0	0	0	+	+			0	0	0	0
FD-11	0	0	0	0	+	0	+	+	+	0	+	0	0	+			0	0	0	0
FD-18	0	+	+	0	+	0	+	0	0	0	+	0	+	+			0	0	0	+
FD-20	0	+	+	+	+	0	0	+	+	0	+	0	+	+			0	0	0	0
Total Spec. Positive	3	5	5	4	3	2	3	3	5	4	4	0	4	4	2	3	0	2	0	1
Total Dogs Positive	6		6		5		4		7		4		5		3		2		1	

*Only four dogs of each group tested. N = nasal - T = throat - R = rectal.

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BACTERIAL FLORA OF BEAGLES EXPOSED TO SR-90 AEROSOLS

by

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INTRODUCTION

To determine the cause of sickness or physiological deterioration in animals exposed to irradiation it is necessary to study many of the measurable changes that occur. These include variations in bacteria normally found in the nose, throat, and gastrointestinal tract. Many such studies have been made of X-irradiated animals but these have been largely concerned with bacteria which can invade from the gut to the circulation. Less attention has been given bacteria of the nose and throat. No studies (to our knowledge) have been made of possible qualitative or quantitative interrelation of bacteria from these areas, in animals which have inhaled radioactive particles.

In a previous report methods were outlined and the frequency of occurrence shown of all bacteria to be found in the nose, throat and lower intestinal tract of a healthy beagle.[1] Methods have been added for the purpose of better identifying the many small gram-negative bacilli found in the throat (Brucellaceae) and the Bacteroides in the intestinal tract.

Eight beagles inhaled particles containing a quantity of Sr-90 which resulted in whole body counts of 31 to 46 microcuries per kilogram. Rectal, nose, and throat swabs were taken on these animals and 8 controls. All dogs were housed in the same room and handled in the same manner. Specimens were obtained before exposure and at 4, 11, 18, and 30 days and at monthly intervals thereafter for 11 months. Results from the first six months will be presented here.

METHODS

The methods of isolation and identification were the same as those outlined previously with the following changes and additions. [1]

Rectal swabs were placed in tubes containing tryptone broth and shaken for 10 minutes. The swabs were removed and the sediment spun down. The supernatant was measured for color or turbidity on a Klett colorimeter. The sediment was then resuspended and diluted to a common standard after subtracting any reading for color. This made an approximately equal amount of fecal material to be inoculated on the plates. A standardized loop was used to make a 1-10,000 dilution and one loopful of this was plated to each of several media. The nose and throat swabs were placed in 1 ml. of trypticase soy broth and then used to inoculate the media. Only the 2 or 3 most numerous organisms were identified, or occasionally as many as 5 if in considerable numbers. It was felt that only those changes involving large numbers of bacteria, where the most numerous on one day were replaced by another species on a succeeding day, would be meaningful in determining the health of the animal.

Since it had been reported that Bacteroides are found in greater numbers in human feces than any other bacterium, including E. coli, an effort was made to find these organisms in the rectal swabs.[2] In our previous study they had been observed frequently. Plates containing neomycin were inoculated and incubated anaerobically in addition to the blood agar plates and thioglycollate broth. [3]

The organisms found in greatest numbers on the chocolate plates streaked with the throat swabs were often small gram negative bacilli which appeared to belong to the Brucellaceae family. In order to have a method for identifying these organisms readily, a set of characteristics derived from a number of authoritative sources[4-7] was used. This scheme is outlined on the following two pages.

Scheme for the Identification of Species in the Families
Brucellaceae, Pseudomonadaceae, and Bacteroidaceae

Features					
C	O	N	I	NA+	NA-
+	+	+	+	P. multocida var. canis P. multocida; P. pneumotropica	H. influenzae; H. parainfluenzae; H. suis; Moraxella H. canis
+	+	+	-	Ps. pseudomallei; B. bronchiseptica; P. hemolytica; P. multocida	H. influenzae; H. parainfluenzae; H. suis; Moraxella
+	+	-	-	B. bronchiseptica	H. parainfluenzae; H. influenzae murium; Moraxella P. novicida
+	-	+	+	P. multocida	H. influenzae; H. suis; H. parainfluenzae
+	-	+	-	A. actinomycet.; B. bronchiseptica; P. pestis; P. psuedotub.; Brucella	H. influenzae; H. suis; H. parainfluenzae
+	-	-	-	B. bronchiseptica; Brucella	S. moniliformis; B. parapertussis
-	+	+	+		H. influenzae; H. parainfluenzae; H. canis
-	+	+	-		H. influenzae; H. parainfluenzae; H. suis
-	-	+	+		H. influenzae; H. parainfluenzae; H. canis
-	-	+	-	A. mallei	H. parainfluenzae; H. suis; H. aphrophilus; H. vaginalis

C = catalas
O = oxidase
N = nitrate
I = indole
NA = nutrient agar

GRAM NEGATIVE BACILLI IN DOGS

Nutrient Agar Positive

	mc	urea	dex.	gel.	malt.	lact.	suc.	cit.
Actino bacillus mallei	-	-	+	-	-	-	-	v
Actino bacillus comycet	-	-	+	-	+	-	-	
Ps. pseudomallei	+	+	+	+	+	+	+	+
B. bronchiseptica	+	+	-	-	-	-	-	+
P. hemolyticus	+	-	+	-	+	v	+	- (hemolytic)
P. multocida	-	v	+	-	v	v	v+	- (non-hemolytic)
P. multocida var. canis	-	-	+	?	-	-	v	
P. pestis	sl	-	+	-	+	+		
P. pseudo-tb	-	+	+	-	+		v	
P. pneumotropica	v	+	+	?	+	v	+	
Brucella	Must be checked with polyvalent serum							

Nutrient Agar Negative

	Satellite	Urea	Dextrose	Gelatin
Hemophilus influenzae	xv		+	Ng
Hemophilus parainfluenzae	v		+	
Hemophilus suis	xv		-	
Hemophilus influenzae murium	x	+	v	-
Hemophilus canis (ovis)	x		+	
Hemophilus aphrophilus	x		+	-
Hemophilus vaginalis	-	v	+	v
Hemophilus species	v	v	-	
Bordetella parapertussis	-	+	-	
Moraxella	-	v	-	v
Pasteurella novicida	-	-	+	Ng
Streptobacillus moniliformis	-		+	-

v = variable

Ng = no growth

RESULTS AND DISCUSSION

Table 1 lists in alphabetical order the organisms found. Thirty-four different species were identified from the nose, 38 from the throat and 24 from the rectum. The organisms found in greatest numbers are listed in Table 2 in the order of frequency with which they were isolated. A comparison with results reported previously on another group of dogs[1] shows that there are some minor changes in the frequency of certain species. This is especially marked in the members of the Brucellaceae family (Bordetella, Pasteurella and Actinobacillus) which were found in much greater numbers in the throat. Clostridium perfringens and Bacteroides were also found more often in the feces. Both of these differences may be due to better methodology in identifying these groups. Although the animals were handled more often and were kept in a different environment, the differences are not great and generally those organisms found in greatest numbers were the same as those found previously. It is pertinent that the frequencies (Table 2) were determined from total numbers of organisms isolated over a period of 6 months while those reported previously[1] were based on per cent of the dogs showing a particular organism and were from one sampling of 25 dogs. The present data were compiled from 11 samplings from each of the 3 sources and from 16 dogs.

There are some differences which may be seen (Table 2) between the number of certain kinds of bacteria isolated from the irradiated and non-irradiated dogs. There were fewer coagulase negative staphylococci isolated over the whole period of study from the noses of the irradiated group. There were more Pasteurella multocida and E. coli isolated from the throats of the irradiated dogs. There were no striking differences in the isolations made from the two groups from rectal swabs where numbers were large enough to have statistical significance. However, there were no coagulase positive staphylococci found in the swabs from the irradiated dogs while 13 were isolated from controls. This may have been due to chance.

A summary of findings of total organisms isolated over the entire

Table 1

Microorganisms Isolated from the Nose, Throat and Rectums of Beagles

Microorganism	Nose	Throat	Rectum
Achromobacter species	+	+	
Actinobacillus actinomycetemcomitans	+	+	
Alcaligenes metalcaligenes	+		
Aerobacter aerogenes		+	+
Bacillus species		+	+
Bacillus subtilis	+	+	
Bacteroides species			+
Beta-hemolytic streptococci Group A	+	+	
Beta-hemolytic streptococci Not Group A	+	+	+
Bordetella bronchiseptica		+	
Bordetella parapertussis		+	
Brucella species		+	
Clostridium perfringens			+
Corynebacterium	+	+	+
Corynebacterium bovis	+	+	
Corynebacterium equi	+		
Escherichia species			+
Escherichia coli	+	+	+
Escherichia intermedium	+	+	+
Flavobacterium			+
Haemophilus species	+	+	
Haemophilus aphrophilus	+		
Haemophilus influenzae		+	
Haemophilus influenzae-murium	+	+	
Haemophilus-like (canis)	+		
Haemophilus parainfluenzae	+	+	
Haemophilus suis (ovis)	+		

Table 1 (Cont.)

Microorganism	Nose	Throat	Rectum
Herellea species			+
Lactobacillus species	+	+	+
Micrococcus species	+		
Moraxella species	+	+	
Neisseria species	+	+	+
Neisseria catarrhalis	+	+	
Neisseria Flavescens			+
Neisseria ova	+	+	
Neisseria (pharyngis group)	+	+	
Nocardia species	+		
Paracolobactrum species		+	+
Paracolobactrum coliforme			+
Paracolobactrum intermedium			+
Pasteurella species		+	
Pasteurella haemolytica		+	
Pasteurella multocida	+	+	+
Pasteurella novicida	+	+	
Pasteurella pneumotropica		+	
Pasteurella pseudotuberculosis		+	
Pseudomonas species	+		
Pseudomonas aeruginosa	+		
Pseudomonas pseudomallei		+	
Staphylococcus, coagulase negative	+	+	+
Staphylococcus, coagulase positive	+	+	+
Streptobacillus moniliformis	+		
Streptococcus faecalis	+	+	+
Streptococcus lactis	+	+	+
Streptococcus mitis (viridans)	+	+	+
Streptococcus sp (anaerobic)			+

Table 2

Organisms Isolated Most Frequently

Number of Times Isolated

Nose				Throat				Rectum			
Organism	Non	Irr	Tot	Organism	Non	Irr	Tot	Organism	Non	Irr	Tot
Streptococcus viridans	46	40	86	Pasteurella multocida	29	43	72	Streptococcus viridans	73	70	143
Coag. neg. staph.	46	33	79	A. actinocomycet.	34	27	61	Cl. perfringens	61	64	125
Neisseria catarrhalis	15	14	29	Streptococcus viridans	30	30	60	E. coli	48	55	103
B. bronchiseptica	11	14	25	E. Coli	17	31	48	Lactobacillus sp.	40	48	88
Corynebacterium sp.	8	16	24	B. bronchiseptica	19	23	42	Streptococcus fecalis	19	13	32
Lactobacillus sp.	8	14	22	Corynebacterium sp.	20	17	37	Bacteroides	15	15	30
H. influenzae-murium	10	10	20	Lactobacillus sp.	17	19	36	Streptococcus lactis	18	9	27
Coag. pos. staph.	8	10	18	Coag. neg. staph.	17	12	29	Coag. neg. staph.	3	18	21
P. multocida	4	8	12	Neisseria catarrhalis	9	12	21	Bacillus sp.	7	12	19
Streptococcus lactis	6	5	11	B-hemolytic strep	11	9	20	Coag. pos. staph.	13	0	13
Moraxella sp.	5	5	10	Neisseria pharyngis	12	7	19	Streptococcus anaerobic	6	5	11

period is shown in Table 3. The bacteria found were grouped according to families because differences within them were not significantly great.

Differences between numbers of dogs in the two groups carrying certain species or groups of bacteria were then determined. Table 4 shows that a member of the family Micrococcaceae was isolated from 33 of the 80 nasal swabs taken from the irradiated dogs and 50 from the 80 control swabs. This is 41% of the irradiated and 63% of the controls, a statistically valid difference at the 5% confidence level.[8] In the same specimens there was a significantly greater number in the irradiated group showing Corynebacteriaceae. Such relationships are worthy of further investigation. Variation in relative number of organisms may be related to the health of the host, as has been observed in humans given antibiotics when the original pathogen may be replaced by large numbers of a commensal which becomes pathogenic.

Table 5 summarized changes seen on a given day of sampling. Only those organisms were included for which there was at least a 50% difference between the experimental and the control dogs. Note that these differences are found almost entirely in the first month, the period when greatest physiological changes have been observed in other species by different methods of irradiation. The alterations indicated in the blocks in Table 5 are not all significant at the 5% confidence level. However, the greater number of Streptococcus viridans in the nose and the throat and the smaller number of staphylococci in the nose of the irradiated group, on the 11th day post-exposure, is significant. So also are the smaller number of staphylococci in the noses of the irradiated group at 25-30 days.

Data similar to those in Table 5 were analyzed only for the two organisms found in greatest numbers. Significant differences were found at identical times for the same organisms isolated from the nose in the two groups. However, differences in the findings for Streptococcus viridans in the throat were just below the 5% level of significance. This is interesting because it suggests that identification of only two organisms per sample may be sufficient to point out real changes in the bacterial flora. This could be of considerable economical importance in a long term study

Table 3

Totals of Organisms Isolated from Irradiated and Non-Irradiated Dogs

Family	Source					
	Nose		Throat		Rectum	
	Irr	Non	Irr	Non	Irr	Non
Pseudomonadaceae	0	2	2	3	-	-
Achromobacteraceae	3	1	7	4	1	-
Enterobacteraceae	1	2	33	21	62	58
Brucellaceae	50	38	140	130	2	2
Bacteroidaceae	0	1	0	0	15	15
Micrococcaceae	45	57	14	18	25	16
Neisseriaceae	23	18	27	31	2	0
Lactobacillaceae	63	69	59	59	145	157
Corynebacteraceae	16	8	17	23	5	3
Bacillaceae	0	1	2	0	76	68
Actinomycetaceae	2	0	0	0	0	0
Totals	203	197	301	289	333	319

8 controls, 8 irradiated with Sr^{90} aerosol. 10 samples taken from each dog and from each source over a period of 6 months. 480 swabs were cultured. A total of 1,642 organisms were identified.

Table 4

Totals of Dogs in Which Organisms were Found

Family	Source					
	Nose		Throat		Rectum	
	I	C	I	C	I	C
Enterobacteriaceae	1	2	26	17	48	41
Brucellaceae	42	36	78	72	2	0
Bacteroidaceae	0	0	0	0	15	15
Micrococcaceae	33	50	10	16	23	16
Neisseriaceae	21	14	25	24	0	0
Lactobacillacae	12	8	52	51	48	39
Corynebacteriaceae	16	6	14	19	3	2

10 samples taken from 8 controls and 8 irradiated dogs from 3 sources over a period of 6 months.

Table 5

Number of Dogs Carrying Bacteria at Different Periods After the Day of Exposure

		Time after exposure									
		Days					Months				
Nose		4	11	18	25-30	36-43	2	3	4	5	6
Strep viridans	Irr	4	(8)	4	2	4	2	6	2	3	1
	Non	5	(3)	3	3	2	5	7	6	5	2
Staphylococci	Irr	4	(0)	(3)	(1)	3	2	5	5	5	5
	Non	4	(6)	(7)	(6)	6	2	5	3	5	6
Corynebacterium	Irr	1	2	2	(4)	2	1	1	1	2	0
	Non	1	1	2	(0)	1	0	0	0	1	0
Throat											
Strep viridans	Irr	4	(2)	1	1	1	4	6	2	2	4
	Non	3	(7)	1	0	0	2	4	2	4	2
Past multocida	Irr	2	1	(5)	5	6	4	1	3	4	4
	Non	1	0	(1)	5	3	6	1	3	2	4
E. coli	Irr	3	1	3	4	(5)	5	0	2	1	2
	Non	2	3	2	1	(1)	2	2	2	1	1
Rectum											
Staphylococci	Irr	0	2	0	6	2	(4)	2	2	3	
	Non	0	0	0	7	3	(0)	3	0	1	
Lactobacilli	Irr	3	2	(1)	6	4	4	8	5	7	
	Non	1	0	(5)	3	2	2	5	7	6	
E. coli	Irr	6	7	5	(8)	6	4	2	3	5	
	Non	7	8	6	(4)	4	3	3	3	3	

There were 8 dogs in each group. If a block (bracket) has 8 appearing in it, this means that all the dogs in the group were carrying this organism. Only those organisms which at some time showed a difference of 4 or more (50 or more per cent) between the irradiated and non-irradiated groups were included in the table above.

involving large numbers of animals.

SUMMARY

A study of the bacterial flora of 8 control dogs and 8 exposed to an aerosol of Sr-90 was made over a 6 month period. There were fewer coagulase negative staphylococci from the noses and more Pasteurella multocida and E. coli isolated from the throats of the experimental dogs over this period. There were significantly fewer of the irradiated dogs with Micrococcaceae and more with Corynebacteriaceae in their noses. The greatest differences in the carrier rate between experimental and control animals were found in the first month after exposure. These were significant for streptococci and staphylococci in the nose and for streptococci in the throat.

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THE SPIROCHETAL POPULATION OF DOGS EXPOSED TO SR-90 BY INHALATION

by

F. F. Pindak

Types and numbers of oral and intestinal spirochetes were determined both in dogs exposed to Sr-90 by inhalation and in controls, at intervals spaced similarly to those in the studies on bacterial and viral flora (see Clapper, et al. and Pindak, et al., this report). The specimens for oral spirochetes were obtained by swabbing the teeth and gum line and the anterior aspects of the tonsils. For intestinal spirochetes, rectal swabs were collected by a procedure described previously.[1] Both types of specimens were submerged in sterile saline and examined by phase contrast microscopy.

In the fecal specimens, the basic three types of spirochetes, characterized earlier[1] were found intermittently in most of the dogs. The oral spirochetes were divided into rigid treponemes and longer flexible treponemes. The borrelias and the double-contoured spirochetes, common in the fecal specimens, were not found among the oral spirochetes. The majority of the flexible treponemes consisted of 10-15 spirals. They exhibited both boring and flexing motion. Among the rigid treponemes, only the boring type of motility was noticed. There was some degree of variability in both the length and the thickness of these organisms and they resembled the treponemes found in the fecal specimens. However, it should not be assumed that the same organism was found in both oral and rectal specimens. The lack of other means than direct microscopic examination prevented further characterization of these organisms.

The numbers of each type of spirochete were counted in ten fields and averaged as follows: none in 10 fields = 0; not more than one per field = +; 2 to 10 per field = 2+; 11 to 50 per field = 3+; more than 50 per field = 4+.

All dogs were examined once before exposure and 6 to 7 times during 180 days thereafter. As can be seen from Tables 1-4, the oral spirochetes were found only occasionally and, generally, in low numbers in both exposed and control dogs. Similarly, no substantial difference in the numbers of spirochetes from fecal samples was found between the two groups.

Results of a recent investigation on intestinal spirochetes[2] indicated that only persistently high numbers of these organisms (11 or more per field), determined by repeated daily examinations, can indicate a possible relationship with disease in beagles. In view of these findings, it was concluded that inhalation of Sr-90 at these dosage levels (31 to 46 microcuries per kilogram) had no effect on the spirochetal population of the digestive tract. At 180 days after exposure the examination of specimens for spirochetes was discontinued.

Table 1 Intestinal and Oral Spirochetes in Dogs Exposed to Sr-90

Dog	Days After Exposure	Intestinal Spirochetes* B	T	D	Oral Spirochetes* T	F
FD-13	2 days before	0	0	2+	0	0
	5	2+	0	2+	0	0
	12	3+	0	+	0	0
	19	+	0	+	0	0
	26	3+	0	+	0	2+
	39	3+	+	0	+	2+
	89	2+	2+	2+	+	+
	180	+	+	2+	0	0
Positive (any degree)		7/7	3/7	6/7	2/7	3/7
3+ or more positive		3/7	0/7	0/7	0/7	0/7
FD-17	2 days before	+	+	+	+	2+
	5	0	+	2+	+	0
	12	0	+	2+	0	0
	19	0	+	2+	+	2+
	26	0	+	3+	+	+
	39	0	+	2+	2+	2+
	89	4+	0	2+	+	+
	180	0	2+	2+	+	+
Positive (any degree)		1/7	6/7	7/7	6/7	5/7
3+ or more positive		1/7	0/7	1/7	0/7	0/7

Dog	Days After Exposure	Intestinal Spirochetes* B	T	D	Oral Spirochetes* T	F
FD-23	3 days before	0	+	2+	+	0
	5	0	3+	+	0	+
	12	0	+	+	+	+
	18	0	0	2+	+	0
	25	0	+	2+	+	3+
	38	0	+	0	3+	2+
	88	0	2+	+	0	0
	179	0	2+	+	0	+
Positive (any degree)		0/7	6/7	6/7	4/7	5/7
3+ or more positive		0/7	1/7	0/7	1/7	1/7
FD-82	3 days before	3+	+	+	+	0
	5	2+	+	+	+	0
	12	3+	2+	2+	2+	2+
	18	+	+	2+	0	0
	25	0	2+	2+	+	+
	38	0	0	2+	2+	+
	88	0	+	2+	0	0
	179	0	2+	+	+	0
Positive (any degree)		3/7	6/7	7/7	5/7	3/7
3+ or more positive		1/7	0/7	0/7	0/7	0/7

*B = Borellia T = treponeme D = double-contoured spirochetes F = flexible treponeme

Table 2 Intestinal and Oral Spirochetes in Exposed Dogs

Dog	Days After Exposure	Intestinal Spirochetes* B	T	D	Oral Spirochetes* T	F	Dog	Days After Exposure	Intestinal Spirochetes* B	T	D	Oral Spirochetes* T	F
FD-14	9 days before	0	2+	2+	0	0	FD-19	10 days before	0	+	2+	0	+
	5	0	2+	2+	0	0		5	+	0	+	0	0
	12	0	+	2+	0	+		11	0	+	2+	+	+
	19	0	2+	2+	0	+		18	0	0	2+	0	+
	32	0	+	3+	0	0		46	0	+	+	+	2+
	51	0	0	2+	0	2+		96	0	2+	2+	+	2+
	142	0	2+	2+	0	0		187	0	3+	0	0	0
Positive (any degree)		0/6	5/6	6/6	0/6	3/6			1/6	4/6	5/6	3/6	4/6
3+ or more positive		0/6	0/6	1/6	0/6	0/6			0/6	1/6	0/6	0/6	0/6
1C	9 days before	0	+	0	0	0	FD-83	10 days before	+	+	2+	0	0
	5	+	2+	2+	0	0		5	+	+	0	0	+
	12	+	+	0	+	0		11	0	+	+	+	0
	19	+	+	2+	0	0		18	0	+	2+	+	0
	32	2+	2+	2+	+	+		46	2+	2+	+	0	0
	51	3+	2+	+	0	0		96	0	+	2+	0	0
	142	0	2+	2+	+	+		187	0	3+	+	0	+
Positive (any degree)		5/6	6/6	5/6	3/6	2/6			2/6	6/6	5/6	2/6	2/6
3+ or more positive		1/6	0/6	0/6	0/6	0/6			0/6	1/6	0/6	0/6	0/6

*B = Borrellia T = treponeme D = double-contoured spirochete F = flexible treponeme

Table 3 Intestinal and Oral Spirochetes in Control Dogs

Dog	Days After Exposure	Intestinal Spirochetes* B	T	D	Oral Spirochetes* T	F
1E	2 days before	3+	2+	2+	0	0
	5	+	2+	2+	+	0
	12	3+	2+	2+	0	0
	19	3+	3+	+	2+	+
	26	4+	+	0	+	0
	39	4+	0	0	0	2+
	89	4+	0	+	0	0
	180	2+	+	+	+	+
Positive (any degree)		8/8	6/8	6/8	4/8	3/8
3+ and/or 4+ positive		6/8	1/8	0/8	0/8	0/8
FD-88	2 days before	0	+	2+	0	+
	5	+	+	+	0	0
	12	+	+	+	0	2+
	19	0	2+	2+	0	0
	26	0	+	+	+	2+
	39	0	+	+	+	0
	89	0	+	3+	0	0
	180	0	2+	0	0	3+
Positive (any degree)		2/8	8/8	7/8	2/8	4/8
3+ and/or 4+ positive		0/8	0/8	1/8	0/8	1/8

Dog	Days After Exposure	Intestinal Spirochetes* B	T	D	Oral Spirochetes* T	F
FD-24	3 days before	0	2+	2+	+	0
	5	0	2+	+	+	+
	12	0	+	+	+	0
	18	0	2+	2+	+	0
	25	0	2+	0	0	+
	38	0	2+	0	+	+
	88	0	+	+	0	+
	179	0	2+	+	+	0
Positive (any degree)		0/8	8/8	6/8	6/8	4/8
3+ and/or 4+ positive		0/8	0/8	0/8	0/8	0/8
FD-84	3 days before	4+	0	+	0	0
	5	+	+	+	+	0
	12	0	+	2+	+	0
	18	0	0	2+	2+	0
	25	4+	+	2+	+	+
	38	0	0	2+	+	+
	88	+	2+	2+	0	0
	179	0	0	+	0	+
Positive (any degree)		4/8	4/8	8/8	5/8	3/8
3+ and/or 4+ positive		2/8	0/8	0/8	0/8	0/8

*B = Borellia T = treponeme D = double-contoured spirochetes F = Flexible treponeme

Table 4 Intestinal and Oral Spirochetes in Control Dogs

Dog	Days After Exposure	Intestinal Spirochetes* B	T	D	Oral Spirochetes* T	F	Dog	Days After Exposure	Intestinal Spirochetes* B	T	D	Oral Spirochetes* T	F
1D	9 days before	2+	2+	+	0	0	FD-18	10 days before	0	2+	2+	0	0
	5	+	+	2+	0	0		5	0	2+	+	+	+
	12	2+	2+	+	+	+		11	0	2+	3+	0	0
	19	0	2+	2+	+	0		18	0	+	2+	+	+
	32	2+	+	2+	+	+		46	0	+	2+	2+	2+
	51	2+	+	2+	0	0		96	0	+	0	0	+
	142	+	0	0	0	+		187	0	0	+	0	0
Positive (any degree)		6/7	6/7	6/7	3/7	3/7			0/7	6/7	6/7	3/7	4/7
3+ and/or 4+ positive		0/7	0/7	0/7	0/7	0/7			0/7	0/7	1/7	0/7	0/7
FD-11	9 days before	2+	+	+	0	+	FD-20	10 days before	3+	+	+	+	+
	5	0	+	2+	0	0		5	+	2+	2+	3+	+
	12	+	2+	+	+	0		11	2+	+	+	+	0
	19	0	+	+	0	0		18	+	+	2+	2+	0
	32	0	2+	2+	+	0		46	2+	+	2+	+	+
	51	+	0	2+	0	0		96	0	0	0	0	0
	142	0	+	0	0	+		187	2+	2+	2+	0	+
Positive (any degree)		3/7	6/7	6/7	2/7	2/7			6/7	6/7	6/7	5/7	4/7
3+ and/or 4+ positive		0/7	0/7	0/7	0/7	0/7			1/7	0/7	0/7	1/7	0/7

*B = Borrelia T = treponeme D = double-contoured spirochetes F = flexible treponeme

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HEMATOLOGIC RESPONSE OF DOGS TO AN INHALED SOLUBLE AEROSOL OF SR-90

by

R. K. Jones

PURPOSE AND METHODS

The purpose of this experiment was to study the biological response of dogs to an inhaled soluble aerosol of Sr-90. The study was designed to investigate a wide variety of biological end points over the life span of the experimental subject. This report, however, deals only with those alterations observed in the hematopoietic system during the first ten weeks post-exposure.

Three baseline hematologic determinations were performed on each animal within two weeks prior to exposure. Parameters investigated were erythrocyte count, hematocrit, hemoglobin, platelet enumeration, sedimentation rate, white blood count, differential and evaluation of the morphologic characteristics of the circulating corpuscular elements. The initial group consisted of 24 experimental and 10 control beagles. All animals were exposed at approximately 13 months of age to a soluble aerosol of Sr-90 and the initial whole body burden ranged from 61 to 200 microcuries per kilogram with a mean for the group of 120.

To investigate initial histopathologic alterations resulting from exposure to Sr-90, two animals and one control were sacrificed at five days and a similar group was studied at 28 days post-exposure. Following the 28th day, therefore, the experimental group consisted of 20 exposed and 8 control dogs. Hematologic sampling was performed at weekly intervals except in the first week during which additional samples were removed on the third, fourth or fifth day post-exposure. Limitations imposed by the number of animals currently supplied through the Project breeding program necessitated a spacing of exposures over a six month period.

Therefore, some experimental subjects have been followed for nine months and others, exposed at a later date, for only three months.

PROGRESS REPORT

The group of animals has been followed for a period of at least 10 weeks and data collected during this period has been selected for inclusion in this report. Information is presented graphically and (unless otherwise stipulated) data points are expressed as the mean per cent of the pre-exposure baseline value.

Total white blood count was lowest at four weeks post-exposure and subsequently rose very slowly through the remaining six weeks of observation (Figure 1). Neutrophils expressed in absolute numbers showed a similar response (Figure 2). The maximum depression of neutrophils, however, was somewhat greater than that of the total white blood cells. Lymphocytes, also expressed in absolute numbers, reached a maximum depression two weeks earlier than neutrophils (Figure 3). Recovery occurred at a slow rate through eight weeks when a secondary fall was seen. No concrete explanation for the curious progressive increase in lymphocytes in control animals can be made but it may be related to the active immunization of all dogs prior to inhalation exposure. This increase in lymphocytes may be a manifestation of immune response in which case it would be anticipated during subsequent observation periods.

Temporally, the depression of white blood cells, neutrophils and lymphocytes was comparable to that obtained in dogs injected intravenously with 100 microcuries per kilogram of Sr-90.[1] The magnitude of depression, however, was considerably less than that observed after the intravenous administration. Whether this is related to differences in per cent retention of Sr-90 or differences in rapidity of deposition cannot be presently ascertained. It is highly significant that a constant disparity of 20 per cent exists between these studies, in total white count, total neutrophil count and total lymphocyte count.

The erythrocyte count, hematocrit and hemoglobin values all showed

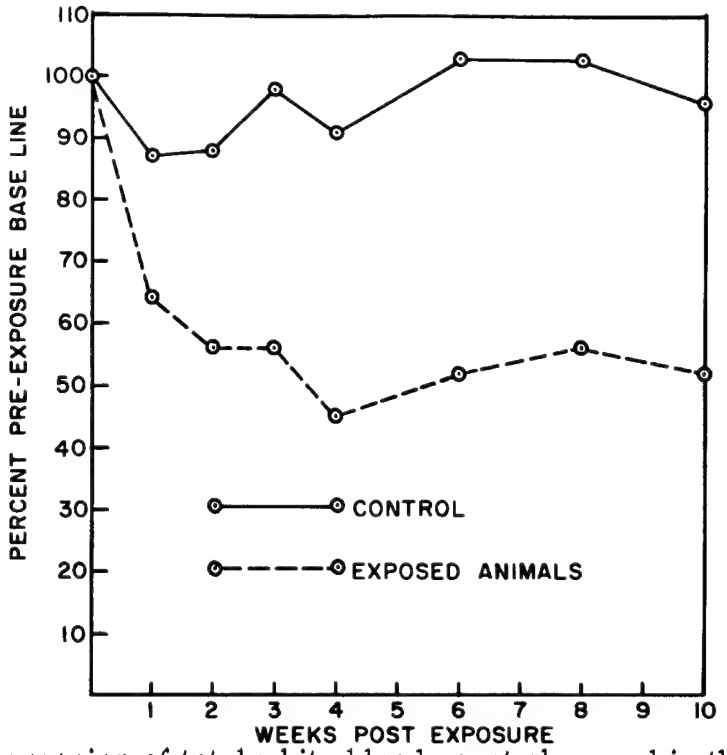

Figure 1: Depression of total white blood count observed in the first ten weeks following exposure to Sr-90.

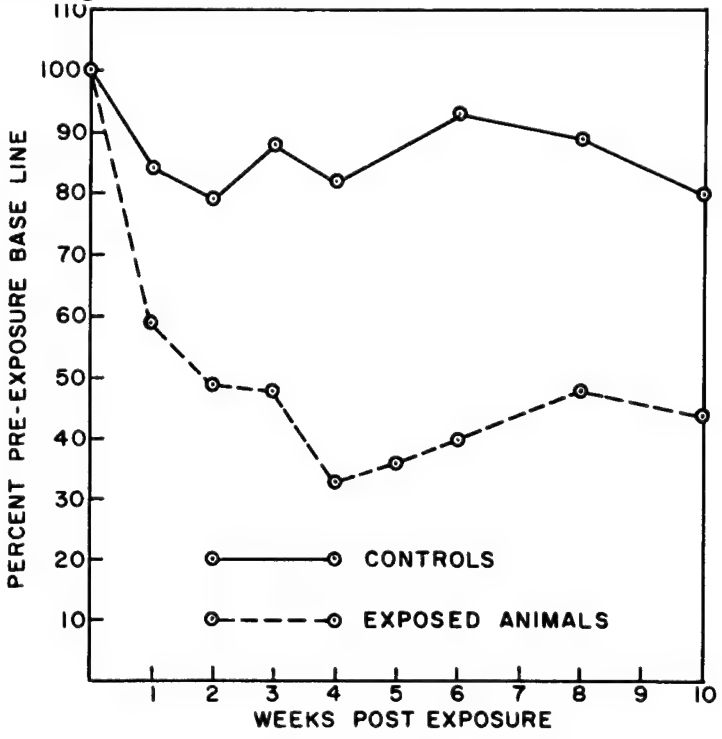

Figure 2: Depression of neutrophil count, expressed in absolute numbers, observed in the first ten weeks following exposure to Sr-90.

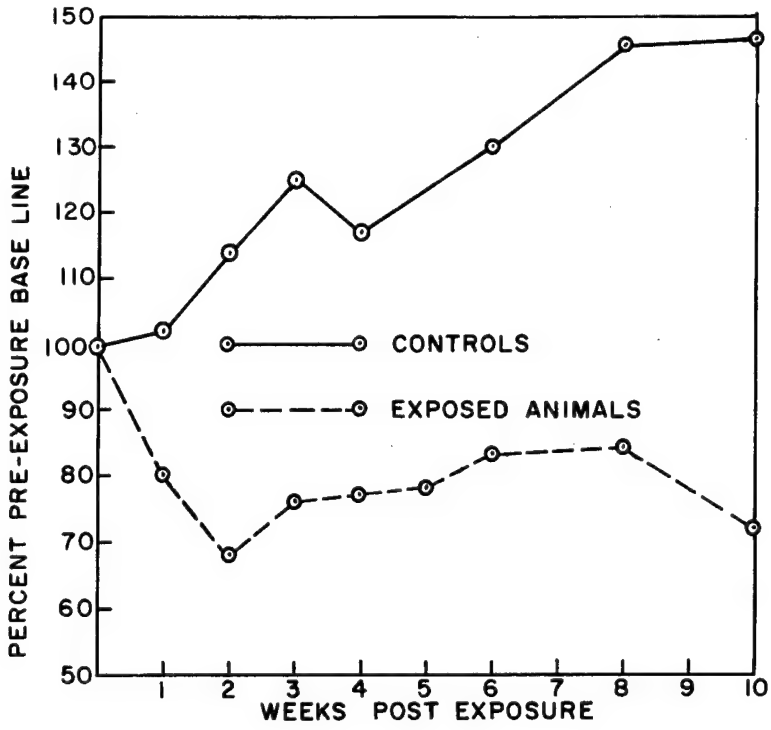

Figure 3: Depression of lymphocyte count, expressed in absolute numbers, observed in the first ten weeks following exposure to Sr-90.

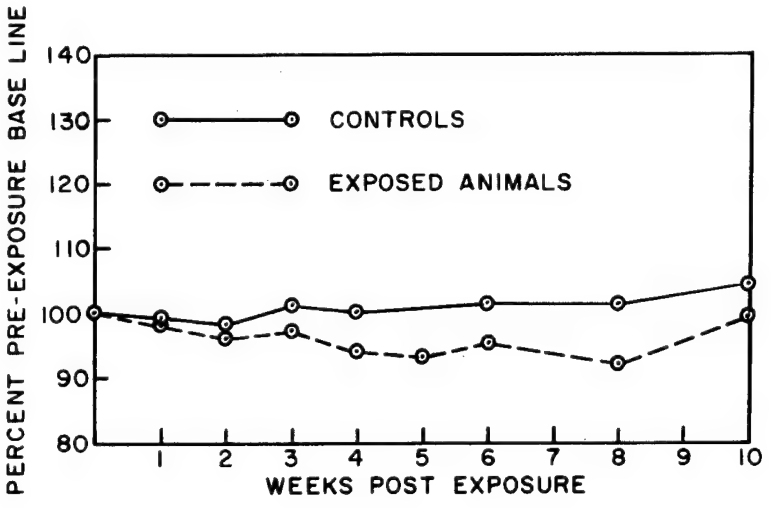

Figure 4: Depression of erythrocyte count observed in the first ten weeks following exposure to Sr-90.

maximum depression between five and eight weeks (Figures 4, 5, 6). The magnitude of depression was essentially similar with all three parameters reaching approximately 90 per cent of pre-exposure baseline values. This also is approximately 10 per cent less than the depression observed in dogs injected intravenously with a similar dose of Sr - 90 but temporally the depression occurred at a similar point. [1]

Maximum depression of platelets occurred four to five weeks post-exposure, reaching a level of approximately 30 per cent of pre-exposure values (Figure 7). There was a rather abrupt recovery to approximately 70 per cent of pre-exposure values by the 10th week. Red and white blood cells were similarly depressed compared to that observed in dogs injected with strontium. However, the magnitude was again approximately 20 per cent less than with injected strontium.

Sedimentation rate became markedly elevated by the fifth week post-exposure followed by an abrupt recovery within the ensuing week (Figure 8). Dougherty and Seymour also described this curious change at approximately one month post-exposure A rather striking difference was seen in the magnitude of this elevation. In their study a value of 25 millimeters per hour was observed at approximately four weeks post-exposure whereas in this group of animals the mean elevation was approximately 7 millimeters per hour.

On the basis of these preliminary data it appears that injury to the hematopoietic system is somewhat less when Sr-90 is administered by inhalation than when observed following intravenous injection. It remains to substantiate this observation and if possible, to delineate the mechanisms responsible for this disparity.

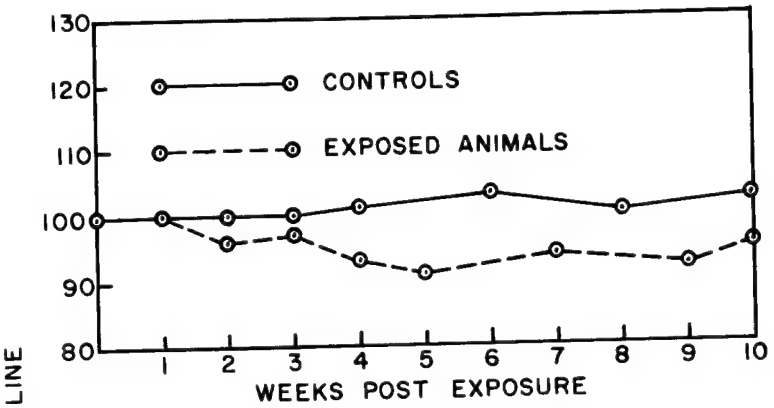

Figure 5: Depression of packed cell volume observed in the first ten weeks following exposure to Sr-90.

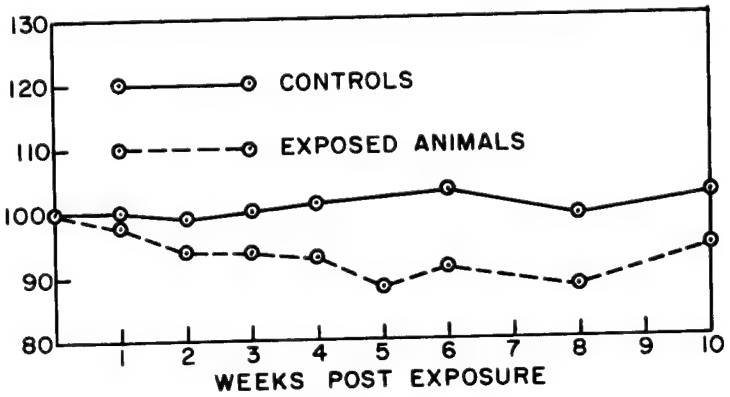

Figure 6: Depression of hemoglobin observed in the first ten weeks following exposure to Sr-90.

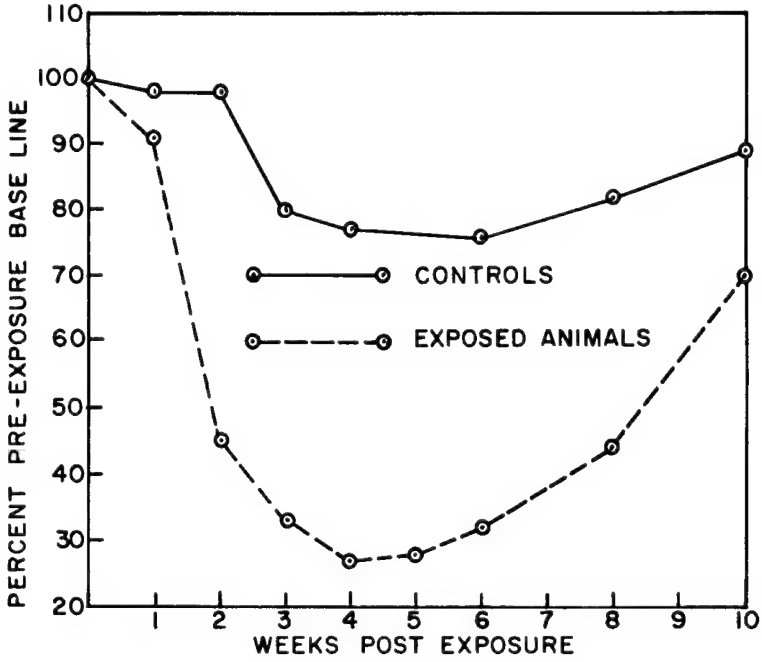

Figure 7: Depression of platelets observed in the first ten weeks following exposure to Sr-90.

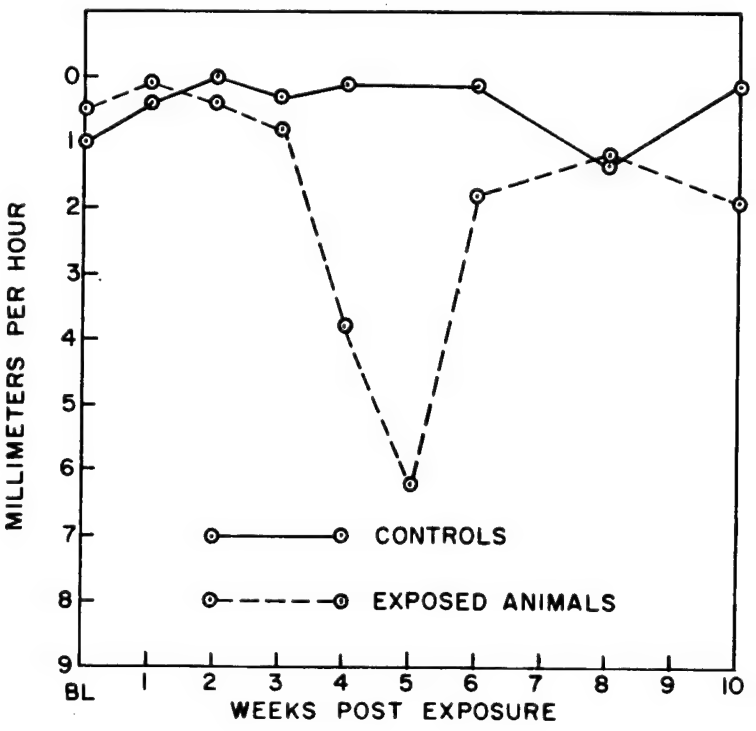

Figure 8: Increase in sedimentation rate observed in the first ten weeks following exposure to Sr-90.

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INDUCTION OF MYELOGENOUS LEUKEMIA IN RATS BY INHALATION OF A SOLUBLE AEROSOL OF SR-90

by

R. K. Jones, J. K. Scott and T. L. Chiffelle

PURPOSES AND METHODS

The induction of leukemia in laboratory animals has proven to be an extremely valuable method for studying the biological behavior of this form of neoplasm. Much useful information has been gathered from research on mice in regard to the leukemogenic effect of radiation and chemical carcinogens, screening of various chemotherapeutic agents and virus transmission of leukemia.

Induction of myelogenous leukemia in rats was first reported in 1937 by De Gennero and Di Grazio, [1] who observed one case among fifteen (15) following cutaneous application of benzpyrine. In 1951 Shay et al. reported induced chloroleukemia in non-inbred Wistar rats fed 20 methylcholanthrene.[2] Subsequently this tumor was successfully transferred to young rats and numerous studies were initiated pertaining to growth and other characteristics of these leukemic cells. The only report of induction of chloroma resulting from exposure to radioactive isotopes was by Zipf and his co-workers who discovered two cases of leukemia among several hundred Sprague-Dawley rats given Actinium 227.[3]

The exceedingly low incidence of spontaneous myelogenous leukemia in common laboratory strains of rats has been well documented.[2-4] To date no spontaneous occurrence has been reported in Sprague-Dawley or Holtzman rats and only three cases have been reported in the Wistar strain.[5] Therefore, the occurrence of even a few cases of myelogenous leukemia probably represents a significant deviation from the norm and it is in this light that the following cases are presented.

PROGRESS REPORT

Incidental discovery of myelogenous leukemia in Holtzman rats was made on a group of animals exposed to a soluble aerosol of Sr-90 and Sr-85. The object was to study induction of malignant bone tumors by serial sacrifice over the life span. Males and females were used and the initial whole body deposition ranged from approximately 100 to 1,000 microcuries per kilogram body weight. Hematologic tests were performed only at sacrifice and therefore no values were recorded for animals that died spontaneously.

The experimental group initially consisted of 120 males and 40 females. However, due to losses on the day of exposure only 110 males and 30 females were available for subsequent study. All animals were either sacrificed or died spontaneously and selected tissues were taken for histologic examination. Although the slide review is by no means complete, there have been four cases of myelogenous leukemia discovered to date. Two cases were diagnosed ante mortum on the basis of abnormalities in peripheral smears, both being subsequently verified by examination of autopsy material. The other two were established only on the basis of autopsy material because they died spontaneously and no peripheral blood was examined.

Although three cases appeared in males and one in a female, no significant sex difference can be established since the ratio of male to female in the experimental group was also roughly three to one. Two distinct forms of myelogenous leukemia were seen. The first represented by two males and one female was of relatively acute nature, characterized by massive infiltration of spleen with myeloblasts and myelocytes. A similar infiltration was seen in portal areas of the liver with occasional groups of cells also present in sinusoids (Figure 1). Ante mortum peripheral blood was available on one of the three animals, the total white count being 101,000 with over 50% being nucleated erythroid elements (Figure 2). Of the remaining cells in the peripheral smear, 10% were blasts unrecognizable as to series type, 2% were myelocytes and the remainder were segmented neutrophils. This animal also displayed a profound anemia with

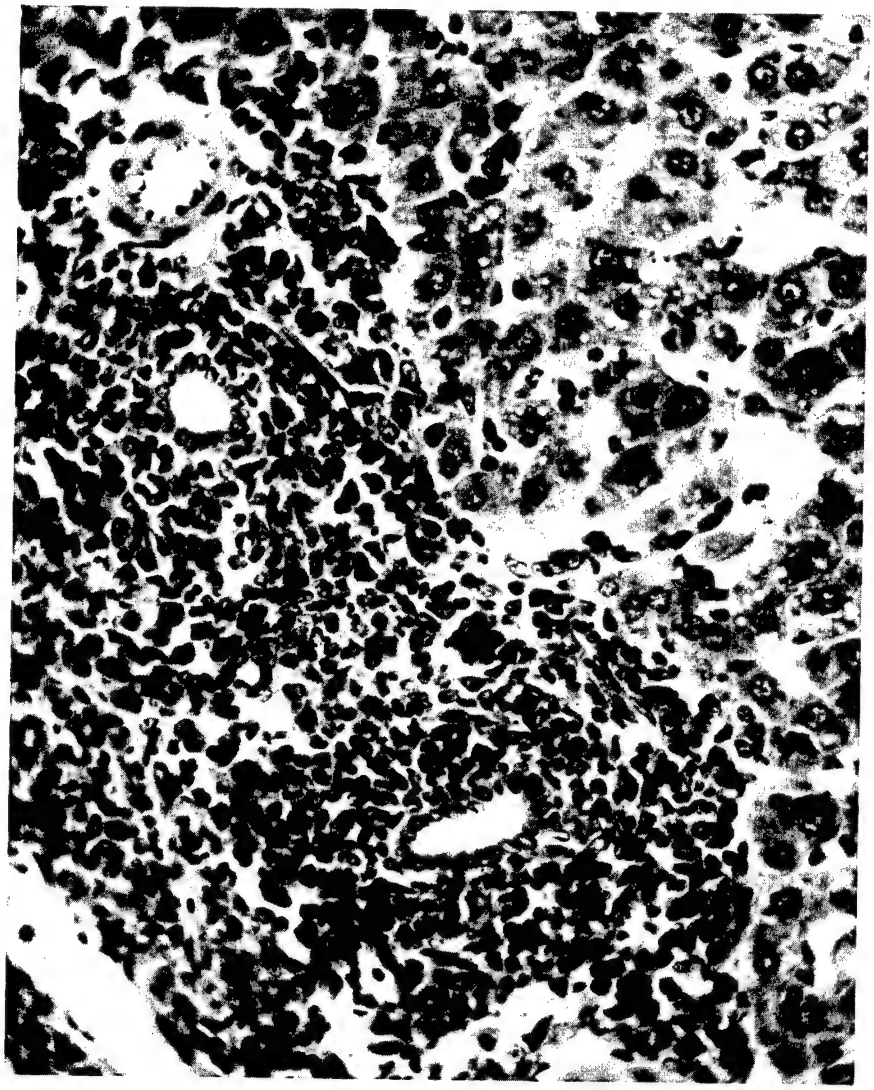

Figure 1: Portal infiltrate consisting of blasts and myelocytes with occasional cells also present in sinusoids (310x magnification).

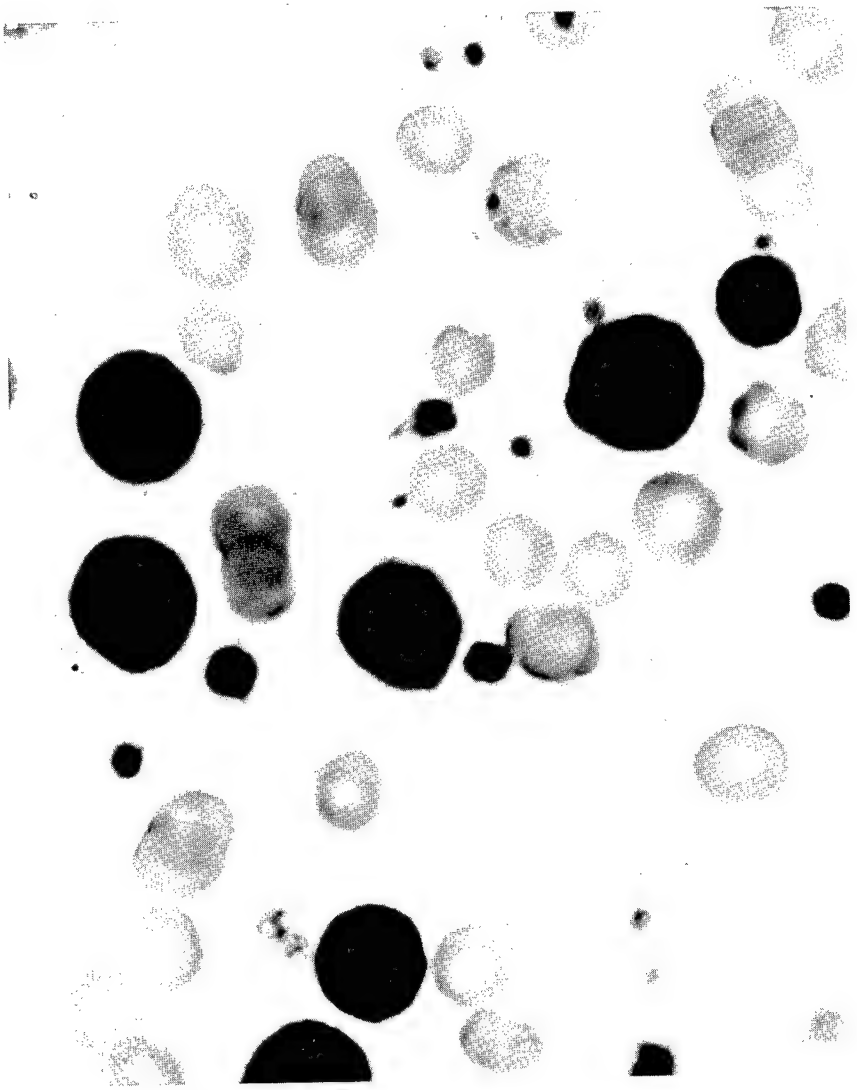

Figure 2: Peripheral blood smear showing nucleated erythroid cells (oil emersion).

a hemoglobin of 2.2 grams. The second form of leukemia was seen in only one animal. Ante mortum peripheral blood was available for evaluation and showed a white count of 671,000. The differential consisted of 10 myeloblasts, 9 myelocytes, 10 metamyelocytes, 16 stabs, 49 segmented neutrophils, 6 lymphocytes and 2 nucleated red blood cells (Figure 3). Hemoglobin on this subject was 11.8 grams with a 34% hematocrit. Autopsy findings were typical of chloroleukemia with green discoloration present in practically all organs. Most notable were infiltrates in the liver, spleen, kidney, bone marrow and lungs (Figure 4). Microscopic examination of these tissues revealed a diffused infiltrate consisting of granulocytic elements in all stages of maturation.

The latent period from exposure to development of leukemia was remarkably constant, ranging from 237 to 279 days (Table 1). Initial whole body deposition ranged from 490 to 780 microcuries per kilogram. Since a direct causal relationship between Sr-90 and leukemia could not be proven with certainty in these four cases, an additional study is contemplated in which equal number of males and females will be exposed to similar doses of Sr-90 and attempts will be made to transplant all subsequent cases of leukemia to recipient weanling rats to study the histochemical, cytogenetic and electron microscopic characteristics of this form of neoplasm.

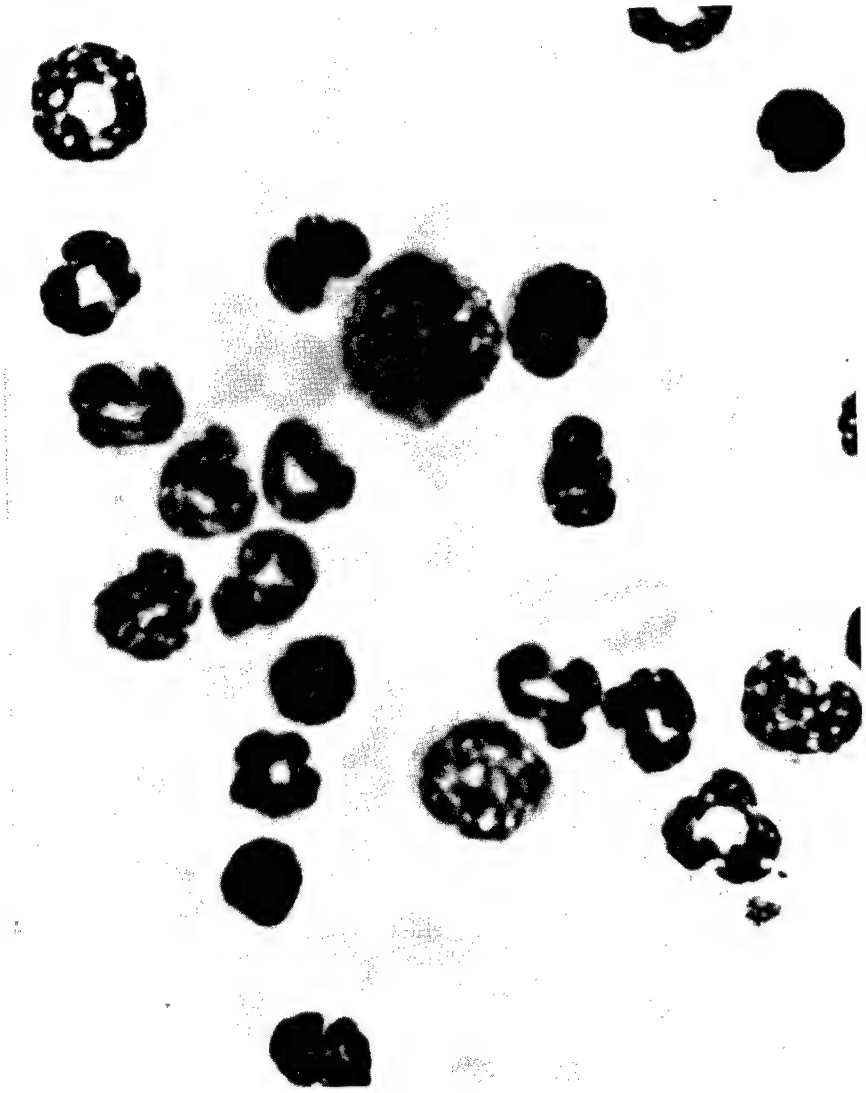

Figure 3: Peripheral blood smear showing young granulocytic elements (oil emersion).

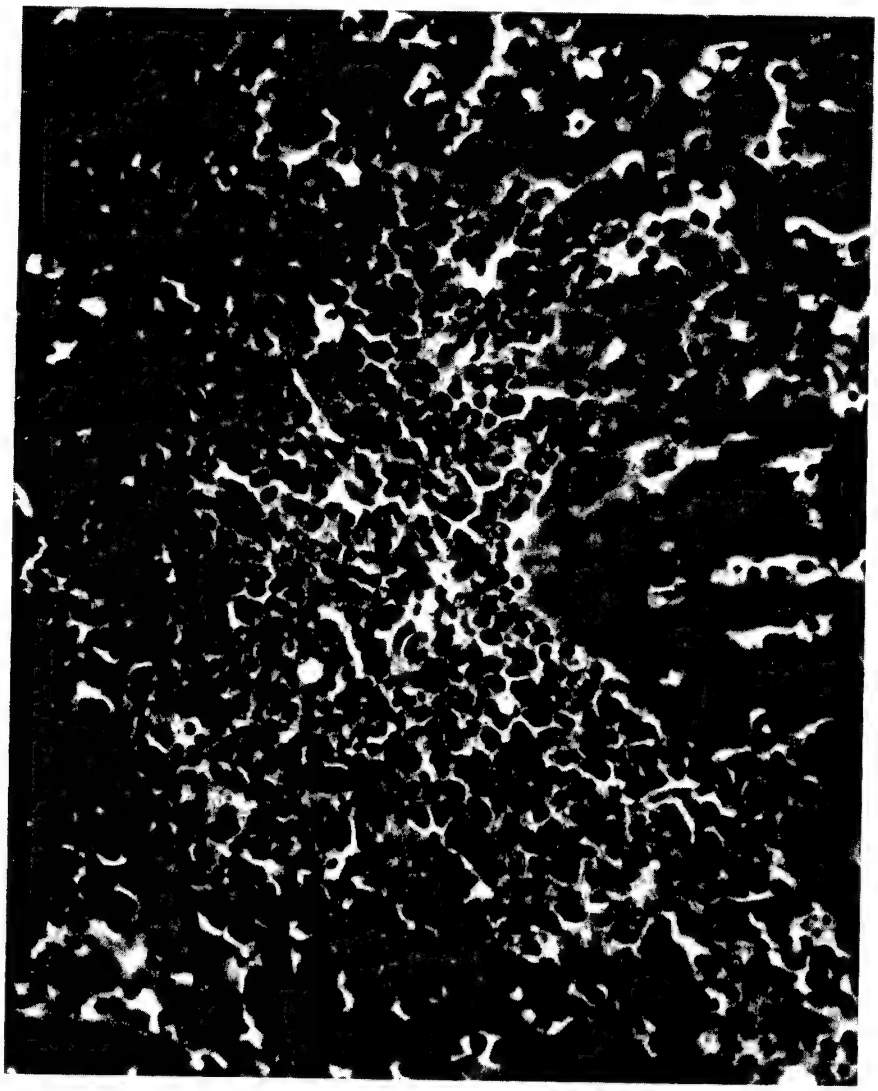

Figure 4: Diffuse hepatic infiltrate consisting of myelogenous cells in all stages of maluration (310 x magnification).

Table 1

Animal	Sex	Age at Death (in days)	Post Exposure (in days)	Dose μc/kg	Type of Leukemia
58-143	M	278	237	650	Sub acute Granulocytic
56-134	F	330	279	780	Sub acute Granulocytic
58-135	M	289	253	700	Chloroma Chronic Granulocytic
66-142	M	278	238	490	Sub acute Granulocytic

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APPENDIX I

Publication of Technical Reports

LF-1 "On the Calibration of Cascade Impactors," T. T. Mercer: September 1962.

LF-2 "The Flora of Healthy Dogs. I. Bacteria and Fungi of the Nose, Throat, and Lower Intestine," W. E. Clapper and G. H. Meade: September 1962.

LF-3 "Ultramicro Methods in Biochemistry. I. General Considerations. II. Procedures for the Determination of Serum Bilirubin," E. Van Stewart, Charles R. Puckett and Agnes Wood: October 1962.

LF-4 "The Tissue Distribution and Excretion of Cesium-137 Following Inhalation, Preliminary Data for Rats," J. F. Stara and R. G. Thomas: April 1963.

LF-5 "A Cascade Impactor Operating at Low Volumetric Flow Rates," T. T. Mercer, M. I. Tillery and C. W. Ballew: December 1962.

LF-6 "Operating Characteristics of the Lauterbach and Dautrebande Aerosol Generators," T. T. Mercer, M. I. Tillery and M. A. Flores: February 1963.

LF-7 "An Electrostatic Precipitator for the Collection of Aerosol Samples for Particle Size Analysis," T. T. Mercer, M. I. Tillery and M. A. Flores: July 1963.

LF-8 "Flora of Healthy Dogs. II. Isolation of Enteroviruses from Lower Intestines," W. E. Clapper and F. F. Pindak: June 1963.

LF-9 "The Particulate State of Fission Products Released from Irradiated Uranium When Heated in Air. I. Preliminary Results for I-131, Te-132 and Ru-103," John C. Gallimore and T. T. Mercer: June 1963.

LF-10 "The Development of a Continuous Cell Line from Normal Dog Liver and Its Susceptibility to Viruses," F. F. Pindak and W. E. Clapper: August 1963.

LF-11 "Procedures and Equipment Used in Inhalation Studies on Small Animals," R. G. Thomas and R. Lie: September 1963.

LF-12 "The Stage Constants of Cascade Impactors," T. T. Mercer: October 1963.

LF-13 "Data Procurement and Processing for the Fission Product Inhalation Program Using an Automatic Read Out Counting System," R. M. Goodrich and R. G. Thomas: December 1963.

LF-14 "A Gamma Ray Detector of Variable Geometry for Whole Body Counting of Small Animals," R. M. Goodrich, R. G. Thomas and S. R. Wright: July 1964.

LF-15 "The Bactericidal Activity of the Serum of Healthy Beagles," G. H. Meade and W. E. Clapper: June 1964.

LF-16 "The Design of a Canine Inhalation Exposure Apparatus Incorporating a Whole Body Plethysmograph," B. B. Boecker, F. L. Aquilar and T. T. Mercer: October 1964.

LF-17 "Response to Secondary Antigentic Stimulus After Whole Body X-Irradiation in the Beagle," F. F. Pindak, J. F. Stara and W. E. Clapper: September 1964.

LF-18 "Ultramicro Methods in Biochemistry. III. The Determination of Serum Cholesterol. IV. The Determination of Glucose," E. V. Stewart and B. B. Longwell: October 1964.

LF-19 "Flora of Healthy Dogs. III. Incidence and Distribution of Spirochetes in the Digestive Tract of Dogs," F. F. Pindak, W. E. Clapper and J. H. Sherrod: November 1964.

LF-20 "Ultramicro Methods in Biochemistry. V. A Comparison of the Ultramicro Determination of Phosphorus Using N-Phenyl-p-Phenylenediamine or 1-Amino-2-Naphthol-4-Sulfonic Acid. VI. The Determination of Serum Alkaline Phosphatase," E. Van Stewart and Bernard B. Longwell: December 1964.

LF-21 "Transport of Relatively Insoluble Materials from Lung to Lymph Nodes," R. G. Thomas: January 1965.

LF-22 "Ultramicro Methods in Biochemistry. VII. The Determination of Plasma or Serum Urea Nitrogen. VIII. The Determination of Serum Uric Acid. IX. The Determination of Serum Creatinine," E. Van Stewart and Bernard B. Longwell: April 1965.

LF-23 "Experimental Animal Housing for the Fission Product Inhalation Program," H. C. Redman: August 1965.

LF-24 "Management Procedures for Animal Colonies Serving the Fission Product Inhalation Program," H. C. Redman and J. H. Sherrod: August 1965.

LF-25 "Experimental Infection of Beagles with Echo Virus Type 6," F. F. Pindak and W. E. Clapper: May 1965.

LF-26 "Susceptibility of Tissue Culture of Canine Origin to Viruses," Frank F. Pindak and William E. Clapper: August 1965.

APPENDIX II

Publications in the Literature

1. Mercer, T. T., "On the Calibration of Cascade Impactors," Ann. Occup. Hyg., 6: 1-14, January 1963.

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